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OBSTETRICS

Cordocentesis in Chiang Mai University Hospital : 286 Cases Experience

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ABSTRACT

Objective To evaluate cordocentesis in terms of indications, results, complications and additional information in this specific methods of prenatal diagnosis.

Design Retrospective study.

Setting Department of Obstetrics and Gynaecology, Chiang Mai University.

Subjects Between September 1989 and December 1994, 286 consecutive cordocentesis were performed.

Main outcome measures Pregnancy outcomes and complications.

Results Mean maternal age was 29.4 years old and about half were second pregnancy. The gestational age of the cases were between 17-37 weeks and 128 (44.8%) were performed at 20 weeks' gestation. The most common indication were associated with haemoglobinopathy, i.e. previous Hb Bart hydrops 82 (28.7%), previous beta Thalassemia entity 77 (26.9%) and 43 (15%) were for chromosomal analysis. Sixty-six cases were detected as abnormal and subsequently terminated. The immediate complication such as bleeding from puncture site or fetal bradycardia were very few. The fetal loss rate was 2.8% while the other obstetric complications were comparable to normal population.

Conclusion Cordocentesis is a useful and relatively safe procedure for prenatal diagnosis.

Key words : cordocentesis, prenatal diagnosis

Fetal blood was first obtained from the capillary circulation of the presenting part after cervical dilatation and rupture of the fetal membranes. This transcervical approach is only possible after a commitment to deliver has been made. Fetal blood sampling (FBS) was undertaken for the prenatal diagnosis of severe inherited disease (with a view to pregnancy termination if the fetus was affected) for many years.[1] Recently, a medical approach to the unborn patients has developed and the role of fetal blood testing in fetal medicine is now comparable to its place in neonatal medicine.[2-6] The puncture sites of FBS are at cord insertion, free loop of cord, fetal portal vein and fetal heart. Nowadays, with the development of high-resolution ultrasonography and increasing experience of operators, the most common site used is umbilical cord, so called cordocentesis.[7,8]

Chiang Mai University Hospital is faced with problems of the most common genetic disease in Thailand, Thalassemia. We performed fetal blood sampling some years ago, mainly to help the couples at risk for a Thalassemic child. In this report the indications and methods for performing cordocentesis in Chiang Mai University Hospital are discussed and potential hazards assessed.

Materials and Methods

Between September 1989 and December 1994, 286 consecutive cordocentesis were performed at Chiang Mai University Hospital. Couples were counselled, including the indications for testing, the risk of procedure, the technique, the chance of failure, the possible fetal loss and therapeutic abortion for affected fetus. All procedures were performed with informed consent.

Cordocentesis were through transabdominal approach. They were scheduled at 18-22 weeks of gestation in the cases who booked for delivery at the hospital except in certained situation such as suspected fetal anomaly by ultrasonic scanning or cases of failed amniocentesis. The procedure was carried out in an out-patient setting with the aid of real-time ultrasound scanner to confirm number, the viability, the gestational age, normality, the location of placenta and site for puncture (cord insertion or free loop of cord). The technique mostly used for fetal blood sampling are cordocentesis, only very few cases other sites have been used (fetal portal vein of fetal heart). Cordocentesis were performed with standard aseptic precaution. A 20 or 22 gauge spinal needle was used under local anaesthesia. The puncture sites might be 2 cm away from insertion point or free loop. The first few drops of fetal blood were discarded to avoid maternal cell contamination, 1-2 ml of fetal blood were aspirated into two 1 ml disposable heparinized syringe which were sent to the laboratory. No more than two attempts were performed at each cordocentesis. The pregnant woman was allowed to go home after the procedure without prophylactic antibiotics or tocolytic therapy. Rhesus-negative women were given an anti-D immunoglobulin prophylaxis. The results of cordocentesis were available 1-2 weeks after the procedures. They were all followed and cared for as high risk pregnancies until delivery. The outcomes were recorded, except for the cases that were referred back to their-own doctors.

If the indication was chromosome analysis, the fetal blood were put into two separate culture bottles containing culture medium and antibiotics. The culture bottles were put in 0.5% CO_2

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The results of both chromosome analysis or HPLC and PCR for Thalassemia indications were available 1-2 weeks after the procedure. Of all the 298 procedures, 15.1% had normal karyotype and 46.0% were not affected by Thalassemia, 26.4% were affected by Thalassemia disease, 2.3% had abnormal karyotype and 5.7% had either beta Thalassemia trait, alpha Thalassemia trait or haemoglobin E trait (Table 3).

The parents of affected fetus elected to terminate the pregnancy either in Chiang Mai University Hospital or at their own physicians'

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The gestational age at delivery and obstetric complications are summarized in Table 5 and Table 6 respectively.

Discussion

This report demonstrates the experience of 286 cordocentesis in Chiang Mai University Hospital and reflects the relative safety and reliability of the procedure. The indication for the majority of cases was related to Thalassemia (80.8%) which is the major problem of genetic disease in Thailand, especially alpha-1 Thalassemia hydrops. 69.9% of cordocentesis was performed at 19-21 weeks of gestation. It appears that this period is not hazardous and most useful time for cordocentesis due to the adequate size of umbilical vessels,[9] less difficult, adequate volume of fetal blood can be obtained and minimal risk from termination of pregnancy in the affected cases. The procedure is easier to perform in later months of pregnancy because the umbilical cord is large.[9] It can be performed regardless of the position of the placenta and the cord insertion, but successful earlier cordocentesis have also been reported.[10] Some particular problems should be considered : in the early stage of pregnancy the diameter of the cord is small and it is difficult to puncture, but access to it at that time is easier because of the large quantity of amniotic fluid compared to the volume of the fetus. The fetus can always be easily displaced if it obstructs access to the cord. On the other hand, in later pregnancy the cord is larger and easier to puncture, but in cases of posterior insertion the fetus sometimes obstructs entirely its access. On these occasions and with the limitation by the length of the needle we have, free umbilical cord loop for puncture sites is advisable, and seem to be safe and less difficult compared to the usual site i.e. at cord insertion.

The major difficulties we have encountered were the obesity of some mothers which reduces the quality of the ultrasound picture ; the mobility of some fetuses and the presence of polyhydramnios which makes puncture sites more difficult to access.

The risks of cordocentesis under ultrasound guidance have to be understood. In this study, risks of failure are few, which is similar to the experiences of other investigators.[8] It is possible to carry out a second attempt some hours or some days later.[8] For the risk of maternal blood contamination, in our experience this risk is low especially in the latter part of our series as we gained more experience. Since we do not have the Coulter Channelyzer in the hospital we have to rely on the notification from the laboratory.

The rate of fetal loss with this procedure, including dead fetus in utero and spontaneous abortion (2.8%), is comparable to the other

investigators.[7,8] If only recent year data (1994) was taken into account, the fetal loss rate was 2.3% reflecting the importance of learning curve.

In our series the rates of premature delivery and other obstetric complications were not significantly different from our current obstetric population.[11] In spite of the fact that in some cases growth retardation was the indication for sampling for a rapid karyotyping, no damaging effects have been noticed in the infants.

The major theoretical risk of the procedure was the possibility of fetal exsanguination from the puncture site on the umbilical cord. The 22-gauge needle we used is small enough to be safe but the volume of blood loss is difficult to measure. However, the duration of the bleeding from the puncture site after withdrawal of the needle was clearly visible on the scan and was noted in each case. In our series it was 7% (3 out of 43 cases which have been recorded) bleeding from the puncture site was very short and did not induce fetal bradycardia.

In the past, difficulties and limitaion of fetoscopy had restricted prenatal diagnosis for some well documented diseases. Direct fetal blood sampling with an ultrasound guided needling is simpler and safer than fetoscopy. Larger volumes of blood can be taken, several assays can be performed and sampling can be done until the end of pregnancy which is particularly important in following the evolution of disease or efficiency of the therapy. Cordocentesis allowed us to approach the prenatal diagnosis of diseases acquired during fetal life such as congenital rubella,[5] congenital toxoplasmosis,[12] fetal cytomegalovirus infection[13] or fetal acidosis in specific condltion.[14] In the fetal therapy field we could study the passage of drugs through the placenta and direct fetal drug therapy by venous passage was also possible.[15,16]

In conclusion, cordocentesis itself seemed to be a safe and reliable procedure. However some experience is needed. In our opinion, it is a procedure of choice in the programme of prevention and control of Thalassemia in our present situation where molecular genetic diagnostic technique are not yet widely available.

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The major theoretical risk of the procedure was the possibility of fetal exsanguination from the puncture site on the umbilical cord. The 22-gauge needle we used is small enough to be safe but the volume of blood loss is difficult to measure. However, the duration of the bleeding from the puncture site after withdrawal of the needle was clearly visible on the scan and was noted in each case. In our series it was 7% (3 out of 43 cases which have been recorded) bleeding from the puncture site was very short and did not induce fetal bradycardia.

In the past, difficulties and limitaion of fetoscopy had restricted prenatal diagnosis for some well documented diseases. Direct fetal blood sampling with an ultrasound guided needling is simpler and safer than fetoscopy. Larger volumes of blood can be taken, several assays can be performed and sampling can be done until the end of pregnancy which is particularly important in following the evolution of disease or efficiency of the therapy. Cordocentesis allowed us to approach the prenatal diagnosis of diseases acquired during fetal life such as congenital rubella,[5] congenital toxoplasmosis,[12] fetal cytomegalovirus infection[13] or fetal acidosis in specific condltion.[14] In the fetal therapy field we could study the passage of drugs through the placenta and direct fetal drug therapy by venous passage was also possible.[15,16]

In conclusion, cordocentesis itself seemed to be a safe and reliable procedure. However some experience is needed. In our opinion, it is a procedure of choice in the programme of prevention and control of Thalassemia in our present situation where molecular genetic diagnostic technique are not yet widely available.

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OBSTETRICS

Prostaglandin E_2 Vaginal Suppository for Induction of Labour in Favourable and Unfavourable Cervix

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ABSTRACT

Objective To compare the pregnancy outcome between patients with favourable and unfavourable cervix.

Design Prospective study.

Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University Hospital.

Subjects Twenty-seven, term, pregnant women were admitted for induction of labour with prostaglandin E_2 (PGE_2) vaginal suppository (3 mg).

Main outcome measures Mode of delivery, time from initial application of PGE_2 until delivery, any adverse effects, Apgar score and immediate newborn status.

Results Caesarean section was performed in 3 out of 13 and 3 out of 14 in patients with favourable (group 1) and unfavourable cervix (group 2) respectively. The mean time of application of prostaglandins to labour (A-L), application to delivery (A-D) and rupture of membranes to delivery (R-D) in cases of successful vaginal delivery, showed no statistical difference between the two groups (group 1 vs group 2, A-L 9.77 ± 7.39 hr vs 12.07 ± 9.02 hr ; A-D 19.45 ± 10.26 hr vs 20.87 ± 8.21 hr and R-D 5.65 ± 5.70 hr vs 6.24 ± 6.82 hr, $P > 0.05$, respectively). No adverse effects occurred both the baby and the mother during the labour period.

Conclusion The pregnancy outcome of induction of labour by PGE_2 vaginal suppository were similar between patients with favourable and unfavourable cervix.

Key words : prostaglandin E_2 vaginal suppository, induction of labour

Medical control of labour is often necessary in modern obstetrics. The status of the cervix may dictate the method of induction and influence its success.[1] Amniotomy and intravenous oxytocin has been used as a standard method for induction of labour in some institutes.[2] However, patients with unfavourable cervix are likely to have prolonged labour with all inevitable sequelae.[3] Locally applied prostaglandin E_2 has been widely used, not only to ripen the cervix but also to induce labour.[4] Many studies have proved it to be simple, safe and highly acceptable to patients and obstetricians, particularly in cases in which a simple amniotomy could not be accomplished.[2,5,6] Nevertheless, the question remains as to what is the pregnancy outcome in different cervical status ? To compare the results between patients with favourable and unfavourable cervcal score, we analysed the following prospective study.

Materials and Methods

This prospective study was carried out at the Department of Obstetrics and Gynaecology, Chulalongkorn University Hospital. Following the approval by our Institutional Review Board, twenty seven women were admitted for induction of labour receiving 3 mg of prostaglandin E_2 (PGE_2) vaginal suppository (Prostin E_2, Upjohn). Inclusion criteria before informed consent were singleton pregnancy, vertex presentation, intact membranes, reactive nonstress test and no evidence of fetal distress. Patients with abnormal lie or presentation, premature rupture of membranes, oligohydramnios, previous uterine scars, uterine contraction, history of allergy to prostaglandins or severe medical diseases such as asthma, heart diseases were excluded from the study. The gestational age was estimated by confirmed last menstrual period during early antenatal care or ultrasonic findings that were compatible with the patients' menstrual dates. All procedures were performed in the labour room. Each patient was checked for cervical score and monitored over a period of 30 minutes to ensure that the fetal heart rates (FHR) were normal and there were few or no uterine contractions (fewer than three in 30 minutes). After an evaluation period, 3 mg of PGE_2 vaginal suppository was placed in the posterior fornix. Then, the patients were asked to remain in prone position for at least 1 hour. In the first 2 hours, the patients were closely monitored for abnormal FHR and uterine hyperstimulation. The Bishop score of 5 or less was considered unfavourable and more than 5 favourable. The cervical score was assessed by the same obstetrician until delivery. After the first 2 hours, the patients received standard Chulalongkorn labour care. Amniotomy was performed when cervical dilatation reached 3-4 cm and other conditions for amniotomy were fulfilled, unless membranes rupture spontaneously. Augmentation with oxytocin was done as indicated, using arithmetic-progression method. Route and method of delivery was performed under obstetric indication.

The following indices are used to measure the outcome : time from initial application of prostaglandins until delivery, incidence of uterine hyperstimulation, or other adverse effects, mode of delivery, Apgar score and immediate newborn status. Averaged data were reported as means and standard deviations and compared by unpaired t-test. $P < 0.05$ was considered significant.

Results

The patients' characteristics of the two

Table 1. Patients' characteristics

	Group 1 (N = 13)	Group 2 (N = 14)	P-value
Mother			
- Age (y)	23.85 ± 3.63	26.79 ± 5.45	0.11
- Partiy	0.15 ± 0.38	0.64 ± 0.93	0.09
- Gestational age (wk)	39.85 ± 1.57	39.93 ± 1.94	0.90
- Initial Bishop score	6.69 ± 0.86	4.29 ± 0.91	0.0001*
Newborn			
- Birthweight (g)	3,084 ± 385	3,022 ± 423	0.69

Group 1 = Patients with favourable cervix

Group 2 = Patients with unfavourable cervix

y = year, wk = week, g = gram

Table 2. Indications for induction of labour

	Group 1 (N = 13)	Group 2 (N = 14)
1. PIH	4	4
2. Postterm	2	5
3. Poor weight gain	1	1
4. Fetal anomalies*	1	1
5. Others**	5	3

PIH = Pregnancy induced hypertension

* Anencephaly , Hydrocephalus

** Thalassemia , Haemoglobinopathy , Systemic lupus erythematosus , decreased fetal movement

groups were similar (Table 1), except for the initial Bishop score. The indications for induction of labour in both groups are seen in Table 2.

The pregnancy outcomes between patients with favourable and unfavourable cervix are demonstrated in Table 3.

Considering only the cases of successful vaginal delivery in each group which also had similar patients' characteristics, we found that the mean time-interval from application of prostaglandin E_2 vaginal suppository to delivery (A-D), application to labour (A-L) rupture of mem-

Table 3. Pregnancy outcome

	Group 1 (N = 13)	Group 2 (N = 14)
Mother		
1. Route of delivery		
- Abdominal	3 @	3 #
- Vaginal	10 (76.9%)	11 (78.6%)
- NL	6	6
- F/E or V/E	4	5
2. Augmentation with Oxytocin	7	5
3. Analgesic given	11	10
4. Postpartum complication	0	1 *
Newborn		
1. Sex (Male : Female)	5 : 8	8 : 6
2. Birthweight (g)	3,084 ± 385	3,022 ± 423
3. Apgar score (At 1 min <7)	1	1 **
4. Neonatal jaundice	2	2

@ Fetal distress due to tetanic uterine contraction from oxytocin (1), Failure to progress (1), Cephalopelvic disproportion (1)

Failure to progress (2) , Cephalopelvic disproportion (1)

* Severe preeclampsia

** Stillbirth (hydrocephalus)

NL = Normal labour and delivery, F/E = Forceps extraction,

V/E = Vacuum extraction

Table 4. Mean time from application of prostaglandin E_2 vaginal suppository to delivery, in cases of successful vaginal delivery

Time in hours	Group 1 (N = 10)	Group 2 (N = 10)*	P-value
1. Application to labour	9.77 ± 7.39	12.07 ± 9.02	0.54
2. Application to delivery	19.45 ± 10.26	20.87 ± 8.21	0.74
3. Rupture of membranes to delivery	5.65 ± 5.70	6.24 ± 6.82	0.84

* Not included one case of hydrocephalus

branes to delivery (R-D) between the two groups were not statistically significant (Table 4).

Discussion

The state of cervix is an important predictor of success in the induction of labour. A firm and rigid (unripen) cervix increases the likelihood of failed induction or prolonged, exhausting labour.[7] In 1987, the guidelines of the American College of Obstetricians and Gynecologists state that a cervical score of at least six is considered favourable and is more likely to result in a successful labour induction.[8] However, while the process of natural cervical ripening predicts a successful labour induction, the effects of iatrogenic ripening are less well defined and remain under clinical investigation.[9]

Prostaglandin E_2 vaginal suppository has been shown to be simple, successful and a safe approach to induce labour, particularly in cases of high Bishop score.[10-12] Nevertheless, there are still variety of opinions and results when using prostaglandins for induction of labour in the unripened cervix. Some studies found it was not so impressive,[1,9] but others revealed satisfactory outcomes.[5,13,14]

In the present study, we used prostaglandin vaginal suppository for induction of labour, comparing between patients with favourable and unfavourable cervix. The pregnancy outcome particularly, the percentage of vaginal delivery between the two groups was quite similar, as shown in Table 3. However, in vaginal deliveries although the application to labour time, application to delivery time, and ruptured membranes to delivery time were shorter in patients with favourable cervix, the difference was not significant statistically. A larger controlled clinical study is needed to confirm or refuse this finding.

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OBSTETRICS

Perinatal Mortality Rate Selected in Bangkok Hospitals and Provincial Medical Schools in Thailand : 1991

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ABSTRACT

Objective To ascertain the accurate perinatal death rate, epidemiological informations and causes of perinatal death from the reliable vital statistic hospital-based data during a one year period.

Design Prospective descriptive study.

Setting Fourteen selected hospitals in Bangkok and three provincial medical schools.

Subjects and methods During January - December 1991 study forms were sent to all those participated hospitals and an obstetrician or neonatal intensive care unit personnel collected all informations and returned completed forms to the principal investigator monthly.

Results There were 99,309 births during one year, 684 stillbirths and 421 early neonatal deaths. Crude perinatal mortality rate was 11.26 per 1,000 total births. When excluding lethal congenital malformations crude perinatal mortality rate was 9.7 per 1,000 total births. For the regional studies,the group of hospitals in Bangkok, Khonkaen, and Songkla have crude perinatal mortality rate per 1,000 total births of 10.3, 18.8, 9.6 and 7.5 respectively. Seventy-nine percent of fetal deaths occurred among mother age group of 20-34 years old, low education, low income, no ANC (22.2%) and insufficient ANC (26.5% ANC less than 4 times). For causes of fetal death, 6.2/1,000 births were maceration, 1.7/1,000 births were asphyxia, 1.5 /1,000 births were premature and unknown 0.8 /1,000 births. Causes of fetal deaths were proved by autopsy in 48.7%.

Conclusion From this study, the problems of mother and child care are a reflection of the whole problems in economy, social and the national education. The government should improve the level of national education, increase the national income and institute better social welfare. Campaign of antenatal care programme, proper identification and referral of high risk cases, counselling programmes for premarital couples, prenatal diagnosis and also encourage family planning during post-natal care are all essential. In addition, the inter-departmental conferences among obstetricians, paediatricians, nurses and medical related personnels should be set up to analyse and correct the problems. With all of these integrated settings, the crude perinatal mortality rate could be reduced.

Key words : perinatal mortality, hospital based, Thailand

Perinatal mortality rate from a survey of Ministry of Public Health in 1988-1989, varied from 11-25 per 1,000 total birth.[1-6] The difference of perinatal mortality rate depends on many factors such as definition and criteria in perinatal statistics, population, place, time and socioeconomic status. On the whole the perinatal mortality is on a decline as reported in the annual conference of "Perinatal Health in Thailand : Regional challenges and prospects."[7]

The objective of this study was primarily to ascertain the accurate perinatal death rate, epidemiological informations, and causes of perinatal death from the reliable vital statistic hospital-based data in one year period. The results obtained hopefully can be used for improving and planning for better perinatal health in the future.

Materials and Methods

A prospective descriptive study of perinatal mortality in 14 self-selected hospitals in Bangkok and three provincial Medical Schools were carried out during January-December 1991. Study forms were sent to all these hospitals participating in this study, namely : Siriraj, Chulalongkorn, Ramathibodi, Vachira, Pramongkutklao, Bhumipol, Phrapinklao, Police, Hua Chiew, Charoenkrung-pracharak, Mission, Srinakarin (Khon-kaen University), Songklanakarin, and Maharaj Nakorn Chiangmai hospitals. Neonatal intensive care unit personnel or obstetrician collected informations such as all babies born and deaths in the delivery room and the neonatal care unit within seven days of age. These informations were sent back to the principal investigator at Department of Obstetrics and Gynaecology, Pramongkutklao hospital. Percentage, mean, standard deviation, Chi-square and T-test methods were used in analysing standard data, with significance set at $P < 0.05$.

Results

Perinatal mortality rate : The perinatal mortality rate of 14 hospitals are shown in Table 1. From 99,309 childbirths 684 of which were stillborn and 421 were early neonatal death. The crude stillbirth rate per 1,000 childbirths was 6.9, and the crude early neonatal mortality rate was 4.3, giving the crude perinatal mortality rate of 11.3. For the regional studies (Fig. 1), the group of hospitals in Bangkok, Chiangmai Hospital, Khonkaen Hospital, and Songkla Hospital have crude perinatal mortality rates of 10.3, 18.8, 9.6 and 7.5 respectively. Excluding 143 lethal congenital malformations such as anencephaly,

thanatophoric dwarf, etc., the crude perinatal mortality was 9.70.

Location : There is no difference in crude perinatal mortality rate of the group of hospitals in Bangkok with or without residency training programme (P = 0.3483). Comparing the crude perinatal mortality rate 10.3 per 1,000 childbirths for Bangkok Hospitals to 18.8 for Chiangmai Hospital, this difference is statistically significant (P < 0.001). However, there is no statistical significance when comparing the crude perinatal mortality rate of the Bangkok Hospitals to that of Songkla Hospital (P = 0.1003), and to that of Khonkaen Hospital (P = 0.385) (Table 2).

Time of death : Amongst conditions of perinatal death stillbirths were the highest (61.9%), while fresh death was 7.2%, 0-7 day after delivery was 24.5%, and death after discharge was 0.1% (Fig. 2).

Multiple pregnancy : There were 722 pairs of twins in which 6 were conjoined and the total number of babies were 1,444. There were 8 triplets with 24 babies (Table 3).

Parity : 93.0% of babies who died were from singleton pregnancy (1.0% of single childbirth) where as 7.0% who died were from multiple pregnancies. From 722 pair of twins, 5.5% died. Death from twin pregnancy was 5 times higher than singleton pregnancy.

Anomalies : 71.2% of babies were normal, 7.1% were noted to have anasarca, while 20.8% had congenital malformations. Of 230 anoma-

Table 1. Perinatal mortality rate

Hospital	Early NND	Stillbirth	Total death	Lethal malformation	Total*	Delivery	PMR/ 1,000	PMR/1,000 (Corrected)	Livebirth
01 Mission	1	14	15	-	15	1,606	9.3	9.3	1,590
03 Police	31	49	80	5	74	6,370	12.6	11.6	6,323
04 Pramongkut	21	26	47	12	35	4,577	10.3	6.6	4,553
05 Siriraj	74	153	227	33	194	18,301	12.4	9.7	18,074
06 Chiangmai	57	94	151	11	140	8,017	18.8	11.4	7,923
07 Vajira	37	56	93	10	81	7,663	12.1	8.0	7,605
08 Bhumipol	36	43	79	12	67	9,270	8.5	6.8	9,226
09 Pinklao	10	49	59	3	56	7,183	8.2	7.1	7,140
10 Songkla	6	11	17	3	13	2,163	7.5	6.0	2,152
11 Hauchiew	23	34	57	3	54	5,269	10.8	9.3	5,235
12 Khonkaen	20	31	51	2	49	5,336	9.6	9.2	5,299
13 Chulalongkorn	48	59	58	28	79	10,411	10.3	7.6	10,353
14 Charoenkrung	26	32	56	6	50	5,373	10.4	9.3	5,341
15 Ramathibodi	31	33	64	15	49	7,770	8.2	6.3	7,739
Total	421	684	1,105	143	962	99,309	11.3	9.7	98,553

* Total = Lethal malformations are excluded from total death
NND = Neonatal death

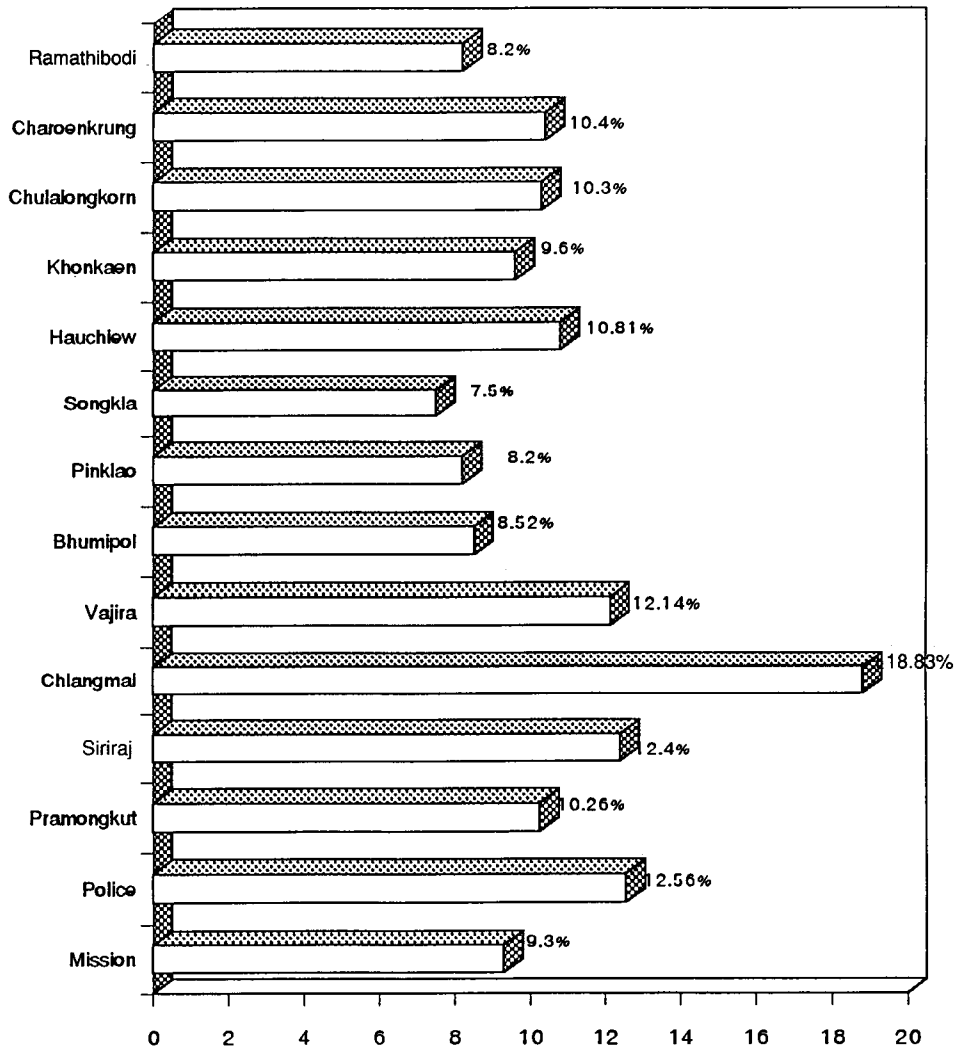

Fig. 1. Perinatal mortality rate.

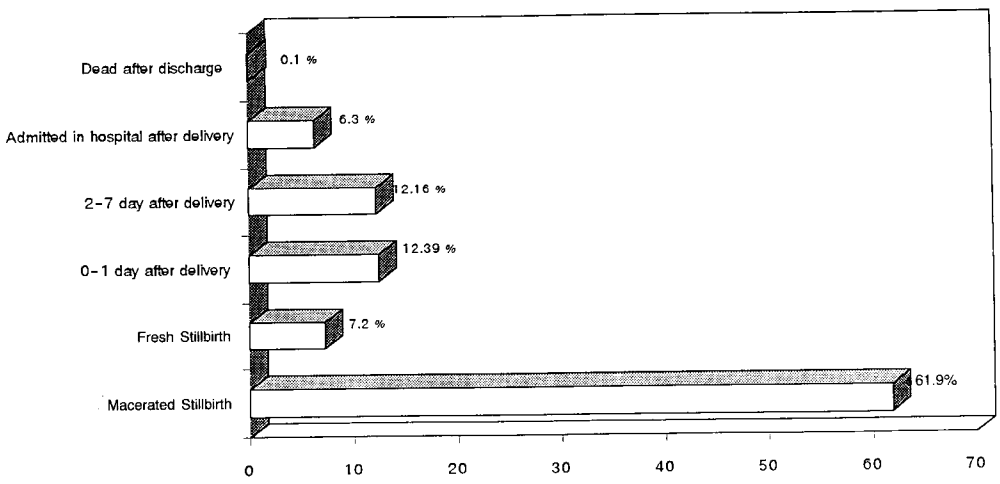

Fig. 2. Time of perinatal death.

Table 2. Comparison of PMR of the group of hospitals in Bangkok to three regional Provincial Medical Schools

No.	Hospitals	PMR/1,000	Compare	P
1.	Hospitals in Bangkok with Residency Training	10.3	1 and 2	0.3483
2.	Hospitals in Bangkok without Residency Training	10.3		
3.	Average PMR/1,000 of Hospitals in Bangkok	10.3	3 and 4	0.001
4.	Chiangmai Medical School (Northern Region)	18.8		
5.	Songkla Medical School (Southern Region)	7.5	3 and 5	0.1003
6.	Khonkaen Medical School (N-E region)	9.6	3 and 6	0.385

Table 3. Delivery of multiple pregnancy

Hospitals	pair of twins	no. of triplet	
Mission Hospital	9	-	
Police	44	1	
Pramongkutklao	35	-	
Siriraj	155	1	1 pair of conjoined twins
Chiangmai	55	1	
Vajira	64	1	
Bhumipol	81	-	
Pinklao	27	1	
Songkla	25	-	2 pairs of conjoined twins
Hauchiew	26	1	
Khonkaen	32	-	
Chulalongkorn	78	2	
Charoenkrung	38	-	
Ramathibodi	53	-	
Total	722	8	3 pairs of conjoined twins

lous babies, 9.4% of them were CNS anomalies, 5.4% had limb anomalies, 3.1% had abdomen anomalies, and 2.4% had spine anomalies.

Birthweights : Infant of low birthweight (< 2500 grams) group contributed 67.6% of death. For weight group between 500-999 grams 5.6% died, 1,000-1,499 grams 24.6% died, 1,500-1,999 grams 17.2% died and 2,000-2,499 grams 20.2% died. Thirty-two percent who died weighed 2,500 grams or more (Fig. 3).

Gestational age : 56.9% of babies who died were at the gestational age less than 36 weeks, 38.7% at the gestational age between 37-41 weeks, and 4.3% were 42 weeks or more (Table 4).

Causes of death : only 48.7% of deaths

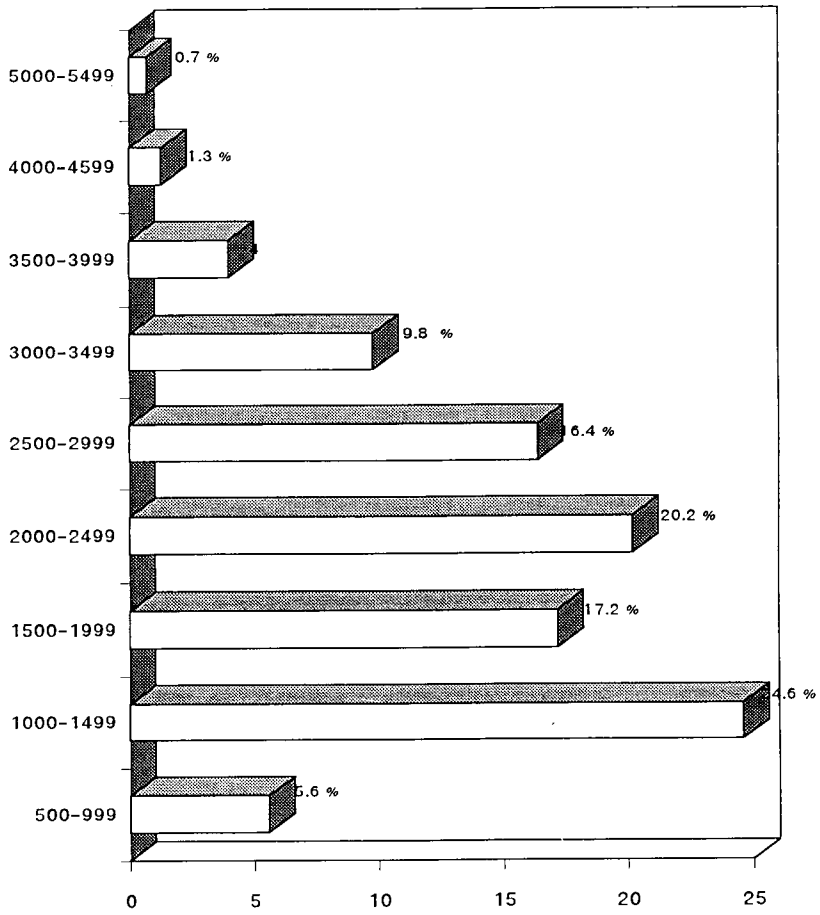

Fig. 3. Birthweights and perinatal deaths.

Table 4. Gestational age of perinatal death

Weeks	Number	%
28-31	270	24.5
32-36	359	32.5
37-41	428	38.7
≥ 42	48	4.3
Total	1,105	100.0

had autopsy. 48.9% of babies were normal but macerated stillborn, 12.9% were macerated with obvious congenital anomalies, 13.9% premature, 15.9% asphyxia, and 8.3% unknown (Fig. 4).

Maternal age : 9.4% of mother had age 15 - 19 years, and 79.0% had 20-34 years (Fig. 5).

Occupation : 39.3% were labourer, 30.8% housewives, 4.6% farmers, 8.9% business, 4.3% government services, and 4.3% unemployed and 7.4% unknown (Table 5).

Education and income : 43% of mothers finished primary school,12.1% completed secondary school, 7.6% finished vocational studies, 3.5% received Bachelor degree, and 0.1% were illiterate, with 33.8% unknown. 18% of families earned 2,000 baht or less per month, 13.5% 2,000 - 3,500 baht per month, 11.8% 6,000 baht or more per month, and 56% were not recorded.

ANC attendance : 22.0% of mothers had never attended ANC, 21.6% attended ANC less than 4 times, and 56.2% attended ANC 4 times

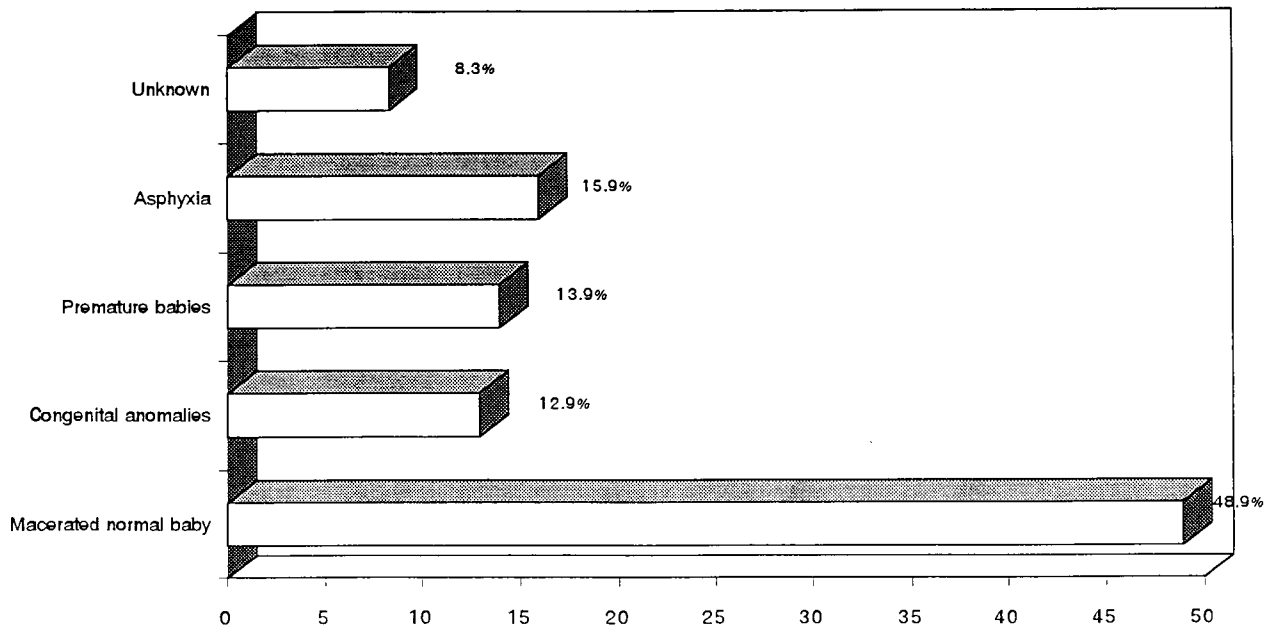

Fig. 4. Causes of perinatal death.

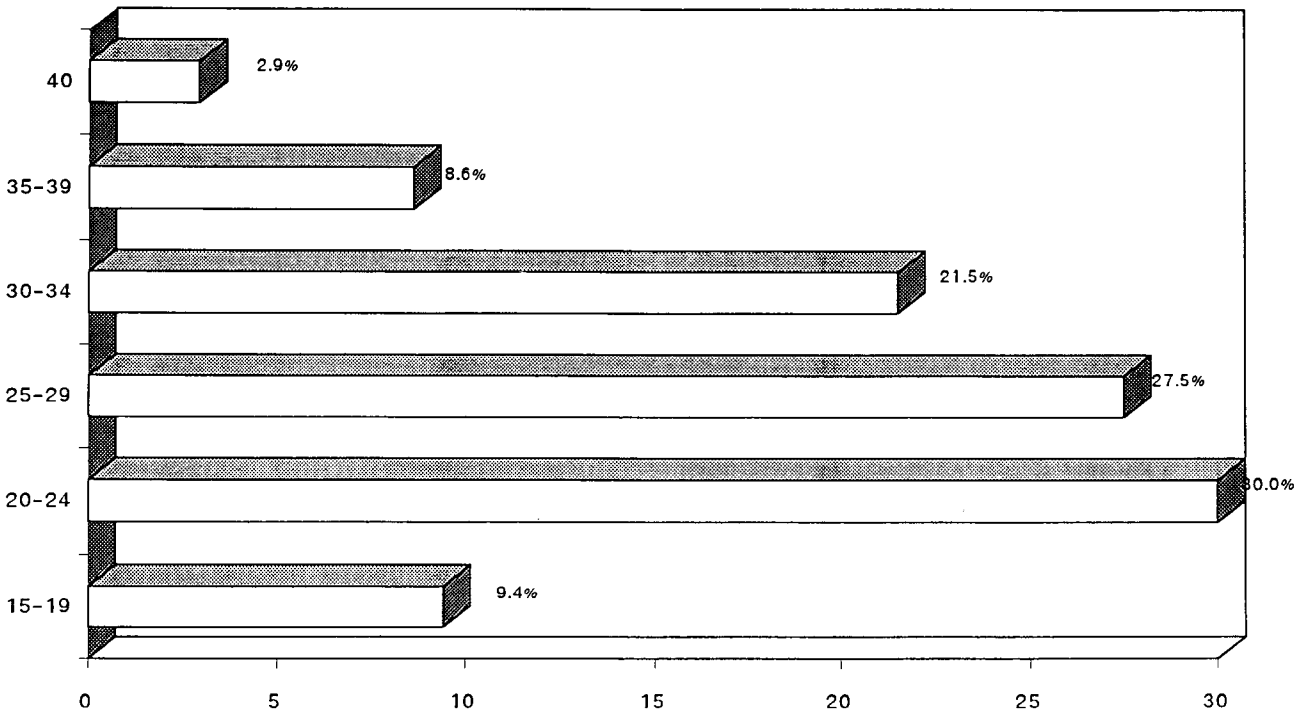

Fig. 5. Maternal age.

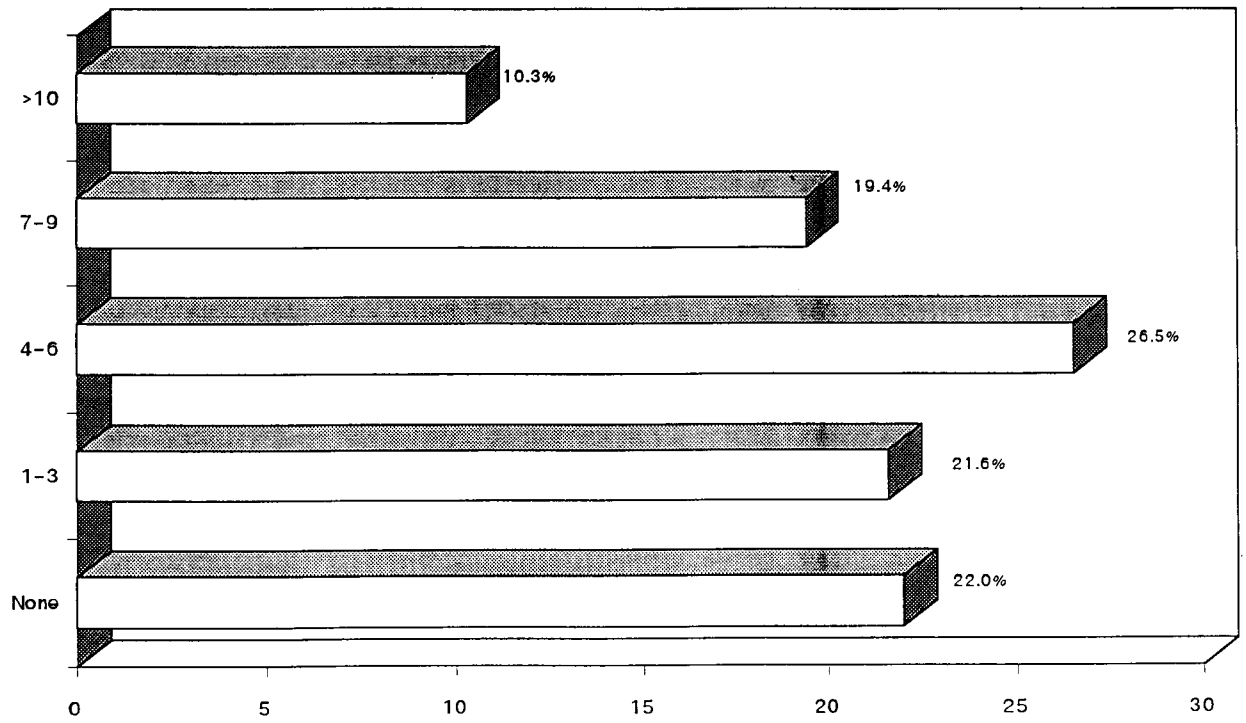

Fig. 6. Number of ANC.

or more (Fig. 6). 19% of mothers attended the first ANC at gestational age of 20 weeks or less, 13.5% attended at 21-27 weeks, 11.0% attended at 28 weeks or more, and 56.3% had no records.

Place of ANC : 31.2% of mothers attended ANC at government hospital, 22.1% at medical school, 7.5% at private hospital, 10.4% at clinic, 4.3% at community hospital, 2.3% at health centre, and 22.1% showed no records (Table 6).

Maternal complication : During pregnancy, 29.0% of mothers had complications. 4.3% had pregnancy induced hypertension, 2.8% hepatitis B virus infection, 1.9% syphilis, 1.4% anaemia, 0.3% heart disease, and 0.2% renal disease.

Type of delivery : 66.3% of mothers had normal delivery, 18.1% caesarean section, 9.0% breech assisting, 3.9% forceps extraction, and 2.7% vacuum extraction (Table 7).

Intrapartum complication : 4.5% had haemorrhage, 4.3% premature rupture of membranes, 1.6% chorioamnionitis, 1.5% prolapsed cord, and 0.2% eclampsia.

Table 5. Occupation of the mothers

Occupation	Number	%
Labourer	434	39.3
Housewife	341	30.8
Business	98	8.9
Farmer	51	4.6
Government Service	48	4.3
Unemployed	47	4.3
Employee	4	0.4
Not available	82	7.4
Total	1,105	100.0

Table 6. Place of ANC

Place	Number	%
Government Hospital	346	31.3
Medical school	244	22.1
Clinic	115	10.4
Private hospital	83	7.5
Community Hospital	48	4.3
Health centre	25	2.3
Not available	244	22.1
Total	1,105	100.0

Table 7. Mode of delivery

Mode	Number	%
Normal	732	66.3
C/S	200	18.1
Breech assisting	100	9.0
Forceps extraction	43	3.9
Vacuum extraction	30	2.7
Total	1,105	100.0

Discussion

The crude perinatal mortality rate (PMR) from this study was 11.3 per 1,000 total births and 9.7 per 1,000 total births when excluding lethal congenital malformations such as anencephaly, multiple anomalies, thanatophoric dwarfs. Comparing PMR amongst different regional hospitals revealed 10.3 in Bangkok, 18.8 in Maharaj Chiangmai Hospital, 7.5 in Songklanakarin, and 9.6 in Khonkaen Hospital. There was statistical difference between PMR in selected Bangkok Hospitals and Maharaj Nakorn Chiangmai Hospital ($P < 0.001$) but no difference in PMR between Bangkok Hospitals and Songklanakarin Hospital, ($P > 0.1003$) or with Khonkaen Hospital ($P > 0.385$). The perinatal mortality in Maharaj Nakorn Chiangmai in the northern part was the highest rate in the study. There were many factors which influence high perinatal mortality such as high maternal mortality rate (63.1/100,000 livebirths), more critical conditions of mothers were referred to the Maharaj Chiangmai Hospital.[8]

The incidence of twin pregnancy in this study was 14/1,000 deliveries of all births. This is higher than other reports.[9,10] Deaths of babies in twin pregnancy were 5 times higher than those in singleton pregnancy. Perinatal mortality occurred more frequently in second twins. Socio-economic factors were the main associated causes of high perinatal loss. These findings were similar to other reports.[10-14] Twenty-two percent of mothers had never attended antenatal care, 21.6% had insufficient antenatal care (less than 4 times). Twenty percent of the mothers had preexisting disease and 29.0% had complications during intrapartum period such as prolapsed cord, PROM more than 24 hours, antepartum haemorrhage and eclampsia. These high risk factors leading to abnormal deliveries, which were 24.8% and contributed significantly to high perinatal mortality.[15-17] For causes of death, 61.9% or 6.2/1,000 were stillbirth (5.2 were macerated, 1.4 were macerated with obvious congenital anomalies). These macerated deaths ranked first in the cause of perinatal death, this is in agreement with several others.[4,6,18,19] Further investigations such as haemoglobin and chromosome study should be instituted in the future study in identifying risk factors. Perinatal mortality in congenital malformation group can be reduced by genetic counselling, prenatal

diagnosis and selective abortion of abnormal fetuses. Sixteen percent or 1.7/1,000 births were deaths from asphyxia and 7.2% or 0.79/ 1,000 births were fresh stillborn that can be used for enquiries into the preventability of perinatal death. Fourteen percent of perinatal death or 1.5 per 1,000 birth were caused by prematurity comparable with 1.4 per 1,000 from other study.[19] As birthweight is a major determinant of perinatal death, birthweight-specific perinatal mortality rate are widely used as an indicator of the quality of health care in pregnancy and perinatal period but this study can not use birthweight-specific rate because of lack of data of weight group of livebirth from each studied hospitals. Autopsy could only be carried out in 48.7% in this study, emphasizing the need for classification that requires no autopsy, i.e. Wigglesworth.

The most effective ways for reduction of perinatal mortality is the Government's action to improve general education, national income and better social welfare, assure providing high quality health care in pregnancy and perinatal period. Premarital counselling and prenatal diagnosis will reduce and eliminate some loss from preventable genetic diseases and congenital malformations. The public must be educated to seek early and continuous antenatal care as well as to demand a quality service during the intrapartum as well as postpartum period.

Acknowledgement

The authors wish to thank representative obstetricians and paediatricians from participated hospitals (Dr. Prassin Chanvitan, Dr. Suraphol Puyanon, Dr. Yupayong Hangchovanith, Dr. Maneesri Laohaviriyakamol, Dr. Pichai Jiaraswadwathana, Dr. Montree Wasinamorn, Dr. Preyapun Saengaroon) who helped in providing clinical informations and vital statistics of this study.

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OBSTETRICS

A Survey of Acceptability of Zidovudine Treatment in Pregnancy Among Thai HIV-1 Positive Parturients

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ABSTRACT

Objective To study the acceptability of zidovudine treatment in pregnancy among HIV-1 positive parturient to reduce vertical transmission.

Design Descriptive study.

Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University.

Subjects Sixty - five cases of HIV-1 positive parturients between November 1994 and December 1995.

Results Fourty - eight cases decided to participate in the zidovudine treatment (73.8%, 95% CI 61.5 - 84.0). Factors which associated with acceptability were gravida and knowledge about vertical transmission.

Conclusion Most of Thai HIV-1 positive parturients accepted to have zidovudine treatment in pregnancy in order to reduce vertical transmission. However, cost-benefit analysis, modified regimens and long term adverse effects should be further studied.

Key words : zidovudine, HIV-1, pregnancy

In Thailand the number of HIV infected mothers have been increased rapidly. The prevalence rate of Thai HIV-1 positive mothers was recently reported as 2%.[1] Strategies to reduce vertical HIV transmission are urgently needed to reduce the number of HIV infected

infants. According to AIDS Clinical Trial Group (ACTG) protocol 076, zidovudine use in pregnancy can reduce vertical transmission rate from 25.5% to 8.3%.[2] United States Public Health Service Task Force has recommended the use of zidovudine to reduce perinatal transmission of HIV.[3] However, zidovudine was rarely used in Thai HIV-1 infected parturients for this purpose. The objective of this study was to survey the acceptability of zidovudine treatment in pregnancy among Thai HIV-1 positive parturients. The result of study could provide information for introducing zidovudine treatment during pregnancy in Thai mothers.

Materials and Methods

Between November 1994 and December 1995, 65 cases of HIV-1 infected pregnant women who attended antenatal care at Ramathibodi Hospital, were interviewed regarding their willingness to participate in the trial of zidovudine use in pregnancy to reduce vertical transmission. All cases were diagnosed during a voluntary screening test for HIV and confirmed with Western blot technique. The zidovudine acceptability questionnaire was administered to HIV-1 infected parturients during post-test counselling session by the authors. The pros and cons of zidovudine use in pregnancy were

Table 1. Some characteristics of HIV-1 infected parturients

Variable	Number	Percent	95% CI
Education			
Illiteracy or primary	50	76.9	64.8 - 85.6
Secondary	8	12.3	5.4 - 12.2
Above secondary	7	10.8	4.4 - 12.0
Occupation			
Housewife	27	41.5	29.4 - 54.4
Business	9	13.8	6.4 - 12.4
Employee	21	32.3	21.2 - 45.1
Farmer	8	12.3	5.4 - 12.2
Family income (per month)			
less than 5,000 Baht	45	69.2	56.5 - 80.1
5,000 and above	20	30.8	19.9 - 43.5
Gravida			
1	29	44.6	32.3 - 57.5
2	19	29.2	18.6 - 41.8
above 2	17	26.2	16.0 - 38.6

Table 2. Age and duration of acceptances and non-acceptances of zidovudine use in pregnancy

Age*	
mean age of acceptance	23.56 ± 3.4 years
mean age of non-acceptance	25.59 ± 5.01 years
Duration of education*	
mean duration of acceptance	6.79 ± 2.32 years
mean duration non-acceptance	6.82 ± 2.33 years

*no statistical difference in both groups

Table 3. Factors associated with acceptability of zidovudine use in pregnancy

Factors	Accepted (n = 48)	Not accepted (n = 17)	P-value
Gravida			
1	25	4	
2	16	3	P < 0.01
> 2	7	10	
Income			
less than 5,000 Baht	32	13	P > 0.5
5,000 Baht and above	16	4	
Occupation			
Housewife	19	8	
Business	9	0	P > 0.05
Employee	14	7	
Farmer	6	2	
Knowledge of vertical transmission			
Yes	42	11	P < 0.05
No	6	6	

informed. The variables of this study composed of age, educational level, occupation, family income, gravidity and knowledge regarding vertical transmission. The descriptive statistics were percentage, mean, standard deviation and 95% confidence interval. Statistical analysis were performed using the x^2 test for proportions and the Student t - test for comparison of means. All data were recorded on to PC microcomputer 486/DX and analysed with statistic package

programme SPSS/PC for Window. Significance is expressed at the 0.05 level.

Results

Between 1st November 1994 and 31st December 1995, sixty-five cases of HIV-1 infected parturients were recruited in this study. The mean age was 24.09 years with standard deviation 3.95 years. Table 1 shows some characteristics of these parturients. Most of them were housewives with family income per month of less than 5,000 Baht and in their first pregnancy. 76.9% had educational level of primary school or lower. 53 cases had knowledge of vertical transmission (81.5%, 95% CI 70.0 - 90.1). Only 2 cases were aware the use of zidovudine in pregnancy to reduce vertical transmission (3.1%, 95% CI 0.37 - 10.7). Responding to questions regarding acceptance of zidovudine use in pregnancy, 48 cases wished to participate in having zidovudine treatment (73.8%, 95% CI 61.5 - 84.0). Considering the factors which might be associated with acceptability of zidovudine use in pregnancy, it was found that age and duration of education made no difference between both groups (Table 2). Table 3 showed some factors which had influenced acceptability. The lower gravidity the more acceptability than higher gravidity ($P < 0.01$). The parturients who had knowledge of vertical transmission were more likely to accept zidovudine use in pregnancy ($P < 0.05$). Income and occupation had no association with zidovudine acceptance.

Discussion

Mother to infant transmission accounts for most of the human immunodeficiency virus infection among children.[3] The ideal approach to reducing perinatal transmission is to prevent HIV infection among women.[3] However, despite on going effort to provide education about HIV prevention, the incidence of infection among pregnant women in Thailand has increased gradually.[4,5] The recently reported results of Acquired Immunodeficiency Syndrome (AIDS) Clinical Trial Group (ACTG) protocol 076 indicated that zidovudine can reduce the risk for HIV vertical transmission by approximately two-thirds[2] and later studies also confirmed these results.[6-10] However, few hospitals in Bangkok have introduced zidovudine treatment in pregnancy. Termination of pregnancy with contraception was the main choice for infected pregnant women.[5] The long term effect of zidovudine on the newborns are not conclusively safe.[11,12] Many women were reluctant to use zidovudine during pregnancy and preferred to have abortion instead. However, very few HIV positive parturients in this study knew about zidovudine use in pregnancy. This study showed some factors which are associated with acceptability of zidovudine among Thai mothers. Age, education, income and occupation had no association with acceptance. However, gravidity and knowledge of vertical transmission were significant factors of acceptance. The lower gravida parturients were more likely to continue pregnancy and accepted zidovudine treatment than higher gravidity because they wished to have children. Knowledge of vertical transmission was also an important factor. The acceptors of zidovudine knew about mother to infant transmission of HIV infection much more than non acceptors. Knowledge of HIV infection is a crucial factor for HIV infected mothers to participate in trials or treatments.[13] Education about HIV infection could encourage the HIV infected mothers to co-operate with doctors and health care providers.

In summary, most of Thai HIV-1 positive parturients accepted zidovudine treatment in pregnancy in order to reduce vertical transmission. The factors associated with acceptance were gravidity and knowledge about vertical transmission. To introduce and encourage the use of zidovudine in pregnancy can reduce number of HIV infected newborns. However, cost-benefit analysis, modified regimens and long term adverse effects are required in further studies.

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OBSTETRICS

Coitus, Masturbation and Sex dream : Prepregnancy and During Pregnancy

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ABSTRACT

Objective To explore the rate and frequency of coitus, masturbation and sex dream prepregnancy and during pregnancy.

Design Retrospective descriptive study.

Setting Department of Obstetrics and Gynaecology, Ramathibodi Hospital.

Subjects One hundred randomly selected normal pregnant women were included between June to December 1992.

Main outcome measures The rate and frequency of coitus, masturbation and sex dream prepregnancy and during pregnancy.

Results During prepregnancy the rate and frequency of coitus, masturbation and sex dream were 100, 54, 28,% and 8.5, 2.8 and 2.4 times per month respectively. During pregnancy in the first trimester these were 82, 28, 19% and 6.0, 1.2 and 1.4 times per month, while in the second trimester 90, 43, 22% and 4.0, 1.7 and 1.2 times per month and in the third trimester 45, 18, 8% and 1.4, 0.6 and 0.8 times per month respectively.

Conclusion The rate and frequency of sexual outlets were markedly reduced and the difference was statistically significant during the third trimester as compared to the prepregnant level.

Key words : coitus, masturbation, sex dream, pregnancy

Many changes of sexual behaviors i.e. coitus, masturbation and sex dream were observed during pregnancy as compared to the prepregnancy period. The influences of the state of pregnancy on these sexual behaviors are not well known. Masters and Johnson found an

increase in sexual tension and performance in the second trimester,[1] but Solberg et al found that for most women coital activity declines in a linear fashion once pregnancy is discovered and also decrease in noncoital behavior, such as masturbation.[2] The difference in findings reflect the highly individual groups of study population that respond to pregnancy. The objective of this study was to explore the rate and frequency of coitus, masturbation and sex dream prior to and during pregnancy among those who delivered at the Department of Obstetrics and Gynaecology, Faculty of Medicine, Ramathibodi Hospital as a baseline information about specific sexual behaviors.

Materials and Methods

This retrospective descriptive study was carried out at the Department of Obstetrics and Gynaecology, Ramathibodi Hospital from June to December 1992. There were 100 randomly selected normal postpartum women who met the following criteria for inclusion in this study : firstly, currently living with husband at least one year before pregnancy. Secondly, there was no psychiatric history or chronic physical disability. Thirdly, voluntary agreement to participate in this study. Each woman was interviewed on the first postpartum day by a female interviewer in a private room. A detailed interview included both open-ended and structured questions. The interview questions consisted of baseline characteristics i.e. her age, husband's age, age at marriage, her education, and gravidity, number and frequency per month of coitus, masturbation and sex dream. For the purpose of analysis, pregnancy was divided into first trimester (1-3 months of pregnancy), second trimester (4-6 months), third trimester (7-9 months), and a baseline of one year prepregnancy.

For statistical analysis, chi-squared with Yates' correction was used to compare coitus, masturbation and sex dream between pre-pregnancy level and during pregnancy (first, second and third trimester). Significance was determined at $P < 0.05$.

Results

The baseline characteristics of one hundred normal postpartum women were, their age ranging form 16 to 42 years at the time of interview, with a mean age of 28.3 (S.D. of 4.2 years). The husband's age ranged from 18 to 43 years, with a mean age of 30.3 (S.D. of 4.8 years). The age at marriage ranged from 15 to 38 years, with a mean age of 23.5 (S.D. of 3.9 years). Distribution of the level of education showed 41% below high school, 24% high school, 22% college and 13% at university level. Obstetric history showed 71% as primigravida, 28% as gravida 2 and 1% as gravida 3.

Prior to pregnancy the rate and frequency of coitus, masturbation and sex dream were 100, 54, 24% and 8.5, 2.8 and 2.4 times per month respectively. During pregnancy in the first trimester there were 82, 28, 19% and 6.0, 1.2 and 1.4 times per month, in the second trimester 90, 43, 22 % and 4.0, 1.7, 1.2 times per month and in the third trimester 45, 18, 8% and 1.4, 0.6, 1.8 times per month respectively (Table 1, 2). The rate of coitus, masturbation and sex dream in the first, second and third trimester showed statistically significant difference when compare to prepregnancy level except masturbation in the second trimester and sex dream in the first and the second trimester (Table 1).

Table 1. Rate of coitus, masturbation and sex dream in each stage of pregnancy and prepregnancy

Type of sexual behaviors	Prepregnancy (%)	First trimester (%)	Second trimester (%)	Third trimester (%)
Coitus	100	82*	90*	45*
Masturbation	54	28*	43	18*
Sex dream	28	19	22	8*

* Statistically significant difference when compared to prepregnancy ($P < 0.0001$)

Table 2. Frequency of coitus, masturbation and sex dream in each stage of pregnancy and prepregnancy per month.

Type of sexual behaviors	Prepregnancy	First trimester	Second trimester	Third trimester
Coitus	8.5	6.0	4.0	1.4
Masturbation	2.8	1.2	1.7	0.6
Sex dream	2.4	1.4	1.2	0.8

Discussion

During pregnancy there are some changes in physical, hormonal and psychological milieu of women but response to these changes varied according to experience in sexual behaviors i.e. coitus, masturbation and sex dream. Many women responded to the changes with a generalized loss of libido. This loss of libido is usually reflected by decreasing sexual behaviors.[3, 4] In this study the finding supported this notion but the decrease was not in a linear fashion as reported by Solberg et al.[2] It was the same as reported by Masters and Johnson[1] and Facilov.[5] This study found that in the second trimester there was an increase in coitus, masturbation and sex dream. The increase in sexual behaviors may probably due to an increase congestion of the pelvic vasculature. In the third trimester, there was markedly reduced in sexual behaviors as similarly reported by Solberg et al.[2] The major reasons for changes may be due to loss of sexual desire, physical discomfort, awkwardness of having sexual activities, fear of injury to baby, sexual desire of the husband[6] and/or recommendation by the physician but this study was not intended to explore these reasons.

In our society, it is possible that details of

coitus, masturbation and sex dream were obscured during interview, however, in this study the rate of respondents' acceptance was very high, all women were very cooperative and responsive so that minimal recall bias would be excluded. In this study informed consent was received from the women themselves, none refused to participate nor discontinued the interview once it began.

In conclusion, besides coitus, pregnant women had masturbation and sex dream as an alternative sexual outlet. The rate and frequency of sexual behaviors were markedly reduced during the third trimester when compared to the prepregnant level.

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GYNAECOLOGY

Adjuvant Chemotherapy for Stage I Uterine Sarcoma

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ABSTRACT

Objective To ascertain the long term outcome for women with stage I uterine sarcoma.
Design A retrospective analysis of case records.
Setting Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital.
Subjects Sixteen women with stage I uterine sarcoma admitted for treatment between January 1982 and December 1987.
Results All of the patients after receiving complete surgery were given adjuvant treatment either chemotherapy or radiation. A follow up period of 60-110 months after surgery was done in all cases. Four cases who received adjuvant radiation therapy all developed recurrence within 18 months after radiation therapy. Another 12 cases were given combined chemotherapeutic drugs of Cisplatin, Adriamycin and Cyclophosphamide, 11 out of 12 cases are alive with no evidence of disease at present while only one patient developed recurrence at 28 months.
Conclusion The study supports that adjuvant chemotherapy could decrease recurrent rate and prolong survival time in this group of patients.

The uterine sarcoma is a malignant tumour arising from either the myometrium or endometrium. From the clinical point of view, it is classified into three major groups, namely leiomyosarcoma, stromal sarcoma, and mixed mesodermal sarcoma. This classification has advantages since it includes almost all uterine sarcoma and avoids the cumbersome. It is a relatively rare tumour of mesodermal origin, encountered in only 2 to 6% of uterine malignancies.[1,2] The management is still controversial because of the rarity of the disease

which hinders prospective studies. Because of a high recurrence rate following surgical treatment in the early stages of the disease, various combinations of adjuvant therapy have been given. Reported herewith a retrospective study of 16 patients with leiomyosarcoma, treated with adjuvant therapy to disclose the role of such treatment on the rate of recurrence particularly in stage I uterine sarcoma.

Materials and Methods

The patients with uterine leiomyosarcoma admitted to the Department of Obstetrics and Gynaecology, Siriraj Hospital from January 1982 to December 1987 were studied. Those with tumour lesions extended beyond the uterine corpus were excluded from this study. The patients were classified employing the Modified Uterine Sarcoma Nomenclature adopted by the Gynecologic Oncology group.[3] All patients, were evaluated following completion of adjuvant treatment and were histologically confirmed to have uterine leiomyosarcoma with the mitotic index of more than 10 in addition the vascular space invasion. After surgical treatment (Total abdominal hysterectomy with bilateral salpingo-oophorectomy) patients were given the adjuvant treatment. The postoperative chemotherapy was a combination of Cisplatin (50 mg/m^2), Adriamycin (50 mg/m^2) and Cyclophosphamide (400 mg/m^2). This was repeated every four weeks for six courses. Those who refused chemotherapy were treated with a 5 week course post-operative radiation of 5,000 rads to the whole pelvis.

After completion of treatment, all patients were followed at 2-monthly intervals during the first year, 3-monthly during the second year and every 6 months in the following years. Complete physical and pelvic examinations were performed at each visit, while chest x-ray examination was carried out every six months.

Results

During the study period, there were 16 patients encountered with stage I leiomyosarcoma. Only 12 patients were allocated to receive adjuvant chemotherapy while four other patients were treated with postoperative radiotherapy.

The follow up period of these patients ranged from 61 to 110 months. Only one patient was found to have recurrent disease 28 months after completion of treatment, as shown in Table 1. The rest of the patients are alive and free of disease.

Four cases also received adjuvant radiation therapy, all developed recurrence within 18 months after radiation therapy.

Discussion

Nowadays there is an increased interest in the role of adjuvant chemotherapy in localized uterine sarcoma as well as metastatic tumour. Since there is not only a relatively low survival rate after surgery in patients with localized uterine sarcoma, and subsequently local recurrence, but also occasionally there is distant metastasis. Adjuvant treatment is therefore believed to be beneficial. Despite adjuvant radiotherapy also being given to patients with this disease, the results are disappointing.[4,5]

Chen reported a high frequency of nodal spread in a stage I uterine sarcoma and it was suggested that lymphatic dissemination might preceed haematogenous spread in early stage of the disease.[6] The increased success rate of adjuvant chemotherapy in this study resulted from two reasons. Firstly, the treatment was given postoperatively to those with only mic-

Table 1. Leiomyosarcoma treated with postoperative chemotherapy

No	Age (years)	MI/10 HPF	Survival (months)
1	49	22	95
2	45	19	28*
3	48	16	110
4	38	22	70
5	47	14	101
6	47	25	71
7	50	18	78
8	51	20	98
9	56	14	68
10	46	20	75
11	46	24	89
12	48	22	61

* Recurrence at 28 months after complete treatment.

MI = mitotic activity

HPF = high power field

roscopic residual disease, thereby greater sensitivity to the drug therapy was achieved, unlike the lesser response rate in the late stage of the disease. Secondly, the combination of Adriamycin and Cisplatin treatment achieved the synergistic effect of a high response rate in this study while single drug response rates from Adriamycin and Cisplatin were only 25 % and 5 % respectively.[7,8] This study demonstrates that adjuvant chemotherapy could eliminate the microscopic residual tumour after surgical treatment, resulting in increased survival rate in patients with early stage of the uterine sarcoma. Although small number of patients were included in our preliminary data, the result showed a significant beneficial effect of adjuvant chemotherapy in stage I leiomyosarcoma. Further study in a larger series of patients should be carried out in order to disclose the appropriate regimen

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GYNAECOLOGY

A Study into the Efficiency of an Ordinary Videocamera and Television when used with a Laparoscope

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ABSTRACT

Objective To develop less expensive equipment as a substitute for the standard videolaparoscope and evaluate its efficiency.

Design Prospective, nonrandomized study.

Setting Department of Obstetrics and Gynaecology, Songkhla Hospital.

Subjects Fifty-three women (experimental) and thirty-three women (control) with the diagnosis of non-malignant ovarian cyst, chronic pelvic pain with dyspareunia, multiparity, unexplained infertility were compared.

Main outcome measures Operating time, amount of analgesics, admission days, and recovery time at home.

Results The equipment was made up from an ordinary home video system (videocamera, television and videorecorder) together with the relevant surgical instruments (laparoscope, air insufflator, light source, trocar and uterine elevator). All this was connected in such a way as to duplicate the functions of the standard videolaparoscope. The experimental group had (1) decreased operating time compared with conventional laparoscopy, (2) decreased use of intramuscular analgesic drugs, (3) decreased number of admission days and (4) required a shorter recovery time at home.

Conclusion The experimental videolaparoscope can be used more comfortably than the conventional laparoscope, it allows the patient to benefit from of minimal invasive surgery as efficiently as the more costly standard videolaparoscope.

Key words : videocamera, laparoscope, pelviscopic surgery

The recent introduction of the videolaparoscope has led to much more efficient investigation and treatment. However, provincial and urban hospitals in Thailand, and may be in other developing countries, have a major problem in that they are often without this equipment because of

its high cost.

Therefore, the author has tried to develop less expensive equipment as a substitute for the much more costly standard videolaparoscope. This equipment was made up from an ordinary home video system (videocamera, television and videorecorder) together with the relevant surgical instruments (laparoscope, air insufflator, light source, trocar and uterine elevator). All this was connected in such a way as to duplicate the functions of the standard videolaparoscope : however, the cost is much less because equipment which is readily available in hospital is used. The efficiency of this experimental videolaparoscope was then evaluated by studying its use in one group of patients and comparing the results with those of another group with which the conventional procedure was used.

Objectives

1. To develop less expensive equipment as a substitute for the standard videolaparoscope.

2. To evaluate the efficiency of this equipment by comparing the operating time, the use of intramuscular analgesic drug, the admission time and the recovery time at home involved in the two procedures.

Materials and Methods

The experimental videolaparoscope was assembled from :

1. A home video system consisting of : a videocamera (Panasonic NV-S8E) ; 14" AV television and VHS videorecorder. Total cost about 40,000 baht.

2. The surgical instrument consisting of : KLI standard diagnostic laparoscope or KLI laprocator, air insufflator, light source, trocar and uterine elevator. All of these elements are readily available in most hospitals although probably used for other purposes.

Both were joined together by a plastic frame specially designed for each type of laparoscope. This frame was made from one-eighth-of-an-inch-thick plastic and glued with ordinary plastic glue. The videocamera was fixed to the frame with a screw and the laparoscope was held in the frame with a rubber "O" ring (Figures 1A and 1B). All equipment was connected as shown in Figure 2 : a videocamera was attached to the eyepiece of the laparoscope, the magnified image projected onto a television screen and the visual documentation recorded on video cassettes.

Its use was studied in fifty-three patients attending the Department of Obstetrics and Gynaecology at Songkhla Hospital from October 1, 1993 to December 30, 1994. The age range was 29-36 (mean 33.87) as shown in Table 1. They were diagnosed for non-malignant ovarian cyst, chronic pelvic pain with dyspareunia, multiparity in need of interval sterilization, unexplained infertility or after non-diagnosis were in need of laparoscopic investigation.

The patients were evaluated to ensure that they were free from any contraindicated conditions for the procedure. After giving their informed consent, they were then diagnosed or

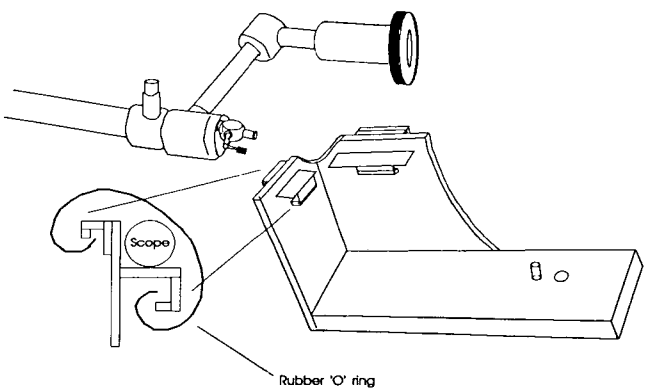

Fig. 1 A. The plastic frame designed for the standard diagnostic laparoscope.

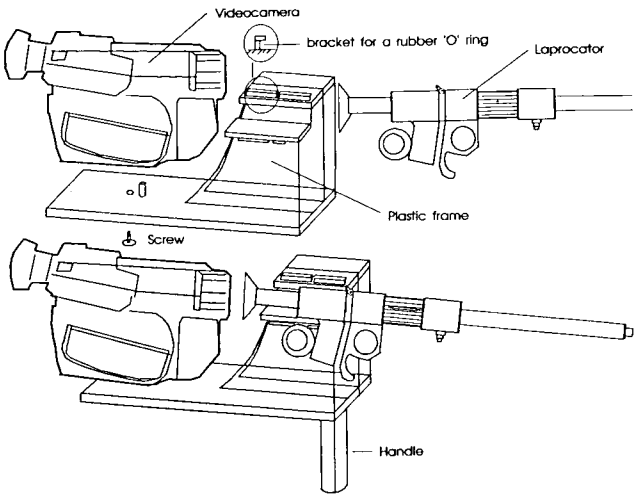

Fig. 1 B. The plastic frame designed for the KLI laprocator.™

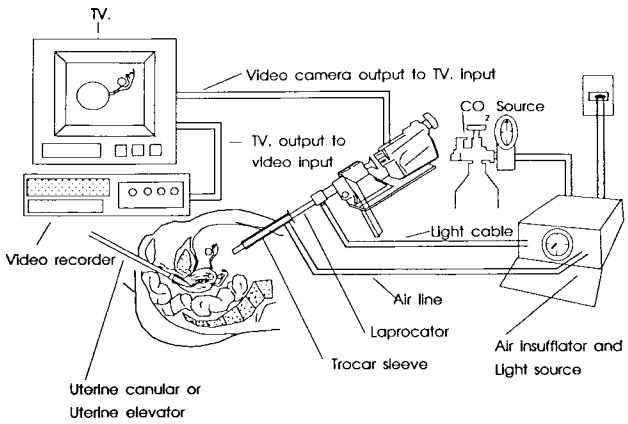

Fig. 2. Video-endoscopic system : interconnection diagram.

Table 1. Mean age (years) for each type of procedure

Procedure	Experimental	Conventional
Salpingo-oophorectomy	36.22 (N=9)	31.82 (N=11)
Uterine suspension	36.40 (N=5)	37.50 (N=3)
Interval sterilization	29.85 (N=13)	31.00 (N=15)
Laparoscopic examination	33.00 (N=26)	27.75 (N=4)
Mean age (Total)	33.87 (N=53)	32.02 (N=33)

treated by using the experimental videolaparoscope. The operating time, the use of intramuscular analgesic drugs, the admission time and the recovery time at home were recorded. The results were compared to those of another thirty-three patients with the conventional procedure.

Results

The comparisons of the operating time, the use of intramuscular analgesia, the admission time and the recovery time at home for each group are shown in Table 2.

Additional advantages resulted, when using the experimental equipment, including benefits for the surgeon as well as the patient, and also the procedure was found to be improved.

Table 3 gives a comparison between the effects of conventional laparoscopy and experimental videolaparoscopy on the user (surgeon) and on the patient : it further shows the differences in the two procedures (both as a technique and as documentation) as well as in instrumentation.

Table 2. Mean operating time, use of analgesia, admission time and recovery time at home

Procedure	Operating time (min.) Exp./Con.	Intramuscular analgesia (dose) Exp./Con.	Admission time (days) Exp./Con.	Recovery time at home (days) Exp./Con.
Salpingo-oophorectomy	90.22 / 47.55	1.10 / 1.82	2.00 / 4.27	20.00 / 30.00
Uterine suspension	62.00 / 60.00	1.60 / 2.50	1.80 / 5.00	16.80 / 23.54
Interval sterilization	14.46 / 19.06	None / None	None / None	5.00 / 5.00
Laparoscopic exam.	18.52 / 27.50	None / None	None / None	8.46 /15.68

Notes
1. All patients, both under the experimental and the conventional groups, were treated by the author.
2. Recovery time at home : the days spent between discharge from hospital to the return to work.
3. The figures in this table are given simply as average value.

Exp. - experimental, Con. - conventional

Table 3. A comparison of conventional laparoscopy and experimental laparoscopy

Conventional laparoscopy (Without monitor)	Experimental videolaparoscopy (With monitor)
User (surgeon)	
1. The surgeon has to work in an uncomfortable position, causing his back and neck to become easily fatigued.	1. The surgeon is able to work in a more comfortable standing position, so that the strain on his back and neck is reduced, facilitating longer procedures.
Patients	
2. It is restrictly used for diagnosis and interval sterilization. When a pathological condition is found, the surgeon must resort to laparotomy.	2. When the final diagnosis has been carried out, the surgeon can proceed to the definite operation through the scope. Thus the patients get benefit from minimally invasive surgery such as reduced intraoperative or postoperative pain, less damage to the abdominal wall, reduced blood loss and shorter hospitalization time.

Procedure	
As a technique	
3. The surgeon peers with one eye through the scope with no magnification, which limits visibility and thus unable to thorough examination of the pelvic organs.	3. The surgeon looks at the television screen. Anatomical structure of the pelvic organs is magnified on the television so that the surgeon is able to appreciate them in greater detail, even small lesions in difficult areas.
4. The assistants cannot see the operative field, so they have to follow the procedure blindly.	4. Both surgeon and assistants can view the operation, enabling the assistants to anticipate the surgeon's needs.
As documentation	
5. Documentation is recorded as a written operative report. It is quite subject to the recollection of the surgeon, or the memory of an assistant.	5. The visual documentation as recorded on video cassettes is easy to be gathered and played back. This promotes education of the surgeon and of other physicians and allows discussion, re-evaluation, and follow-up of disease processes.
Instrumentation	
6. Ordinary equipment and preparation techniques are used.	6. More preparation time is required. The surgeon must understand the laparoscope-video-television system and be able to handle system failure.
7. Standard sterilization techniques are carried out.	7. The techniques to keep the operative field sterile are more difficult. In this study, the author used double glove technique to fix a videocamera to the plastic frame which were then covered with a sterilized plastic bag. Nevertheless, the use of a videocamera could increases the risk of operative field contamination.

Discussion

Recently many studies have emphasized on the improvements of patient care when the videolaparoscope is used instead of the conventional laparotomy in various surgical procedures.[1-6] For example, Hershlag et al stated that laparoscopy should be the preferred surgical approach to the diagnosis and treatment of pelvic adhesions whenever possible because it is more convenient to the patient, less expensive, saves hospital beds and involves low morbidity.[1] Schwartz and Martin demonstrated that the advantages of operative laparoscopic salpingectomy were : decreased morbidity and surgical pain, lower costs, shorter hospitalization and convalescence and less disability, as well as a cosmetic scar.[2] Keye noted that although advances in operative laparoscopy had not yet led to improved pregnancy rates, they had decreased costs and morbidity of surgery for endometriosis.[3] Semm found that hospitalization was reduced by approximately three days and convalescence by approximately one week when laparoscopic techniques was used. He states that for some gynaecological procedures (such as operative treatment of ectopic pregnancy, operations to correct sterility, benign ovarian tumours and the enucleation of myomas of up to 400 grams in weight) laparotomy is now indicated only in rare cases.[4] Wood and Maher[5] and Camran et al[6] also supported these findings. Camran et al also demonstrated that some aspects of laparoscopic techniques can be used in gynaecologic and general surgery.[6]

Although in some parts of the world these techniques are now common by used, the situation is not the same in some developing countries. For example, in Thailand, well-trained surgeons are frequently not able to gain their experiences pass on their patients because of the high cost of the standard equipment (about 1,700,000 baht). This study, therefore, was an attempt to solve the problem by developing a less expensive equipment as a substitute for the standard videolaparoscope. The efficiency of this experimental videolaparoscope was then evaluated by studying its use. The benefits were obtained similarly to those studies using the standard videolaparoscope referred to earlier.[1-6] Comparing the average operating time it was found that the use of the experimental videolaparoscope required a shorter time than that of the conventional laparoscopy for interval sterilization and laparoscopic examination, but no operating time was saved in cases of open laparotomy for salpingo-oophorectomy and uterine suspension. The use of intramuscular analgesic drugs, the days spent in hospital and the recovery time at home were also considerably less. This was because the experimental procedure requires only a small incision and needs no abdominal retractor so there is less manipulation to the pelvic organs. Comparing with the conventional laparoscope was found to be the experimental videolaparoscope more effective because it showed a sharper and wider view of the pelvic organs so that more detailed information were obtained. The surgeon is able to work in a more comfortable standing position, so that the strain on his back and neck is reduced, facilitating longer procedures. Furthermore the assistants are also able to view the operation, so they can help in the precise way the surgeon needs.

Two factors that must be considered in this study were the image resolution and the weight of the equipment, which was approximately one kilogram. Both depend on the type and the quality of the videocamera and of the laparoscope being used. New developing

technology is hope fully leading towards smaller and lighter videocameras of higher quality.

In conclusion, the experimental videolaparoscope (1) can be used more comfortably than the conventional laparoscope, (2) allows the patient to benefit from minimal invasive surgery as efficiently as the more costly standard videolaparoscope, (3) can be used without postoperative complications. The author believes that the use of an ordinary videocamera and television with a laparoscope will increase the quality and efficiency of patient care in developing countries where the standard equipment is not fully available.

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REPRODUCTIVE SCIENCE

Intracytoplasmic Sperm Injection (ICSI) : the First Reported Case of Pregnancy in Thailand

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ABSTRACT

Objective To present preliminary experience on the treatment of male factor infertility by the so-called ICSI technique, and to report the first successful ICSI pregnancy in Thailand.

Design A retrospective analysis of first 12 ICSI-cycles.

Setting Infertility Clinic, Chiang Mai Ram Hospital, Chiang Mai, Thailand.

Subjects Ten consecutive couples with the diagnosis of male factor infertility or with previous failed fertilization in conventional IVF during August 1994 through February 1995.

Main outcome measures Fertilization and ongoing implantation and pregnancy rates.

Results From a total of 100 oocytes collected, 88 were considered mature and recruited for ICSI, 80% of which survived the procedure. Sixty-one percent of the intact ICSI-oocytes (43/70) fertilized normally (2 pronuclei) and most of them (88%) cleaved in vitro. Overall, thirty "good-looking" embryos were transferred in altogether 12 cycles and three pregnancies were obtained, giving the implantation rate of 10% and 25% clinical pregnancy rate per transfer. Consequently, two pregnancies unfortunately aborted at 6-7 weeks of gestation and one successful pregnancy which completed in a healthy preterm male baby.

Conclusion For male factor infertility, ICSI seems to be a very impressive solution with promising results.

Key words : intracytoplasmic sperm injection, ICSI, male-factor infertility, in vitro fertilization, IVF

Intracytoplasmic sperm injection (ICSI) has recently been described as beneficial in alleviating male-factor infertility.[1] With standard in vitro fertilization (IVF), only a small number of male factor problems can be solved. Thus, ICSI would be helpful not only for most of these couples suffering from severe male factor infertility with failed IVF, but also for cases with too low a number of motile spermatozoa in the ejaculate.[2]

This preliminary report describes the experience of our early-stage ICSI programme and the successful result of this innovative assisted fertilization technique.

Materials and Methods

Patient selection

From August 1994 to February 1995, twelve treatment cycles by ICSI in 10 couples were carried out at Infertility Clinic of Chiang Mai Ram Hospital. Previous treatments were carried out in this centre or in centres which referred the couples to this centre specifically to have ICSI. The mean age of the female patients was 30.7 ± 3.9 years (range 26-39 years) and the mean age of their partners was 36.5 ± 4.2 years (range 31-46 years). For the male patients, the majority of them (7 out of 10) had very severe oligoasthenoteratozoospermia (i.e., total motile count of less than one million spermatozoa with limited motility and/or obviously abnormal sperm predominance). The mean concentration of these semen analyses was only 300,000-400,000/ml (range 0.2-0.8 million/ml). The remaining three cases had normal semen analyses (concentrations of 30 to 70 million/ml) with at least one total or almost total fertilization failure in standard IVF cycles.

Patient counselling included information about the novelty of this established assisted fertilization technique. A patient consent form which included prenatal diagnosis by amniocentesis as well as a prospective follow-up of the children born after the ICSI procedure was then signed.

Ovarian stimulation

Ovarian stimulation was carried out by either "long" (desensitizing) or "short" (flare-up) protocol of the intranasally administered gonadotrophin-releasing hormone agonist (GnRHa) ; buserelin (Suprefact) ; in association with urofollitrophin (Metrodin) and human menopausal gonadotrophins (Pergonal or Humegon). After the stimulated follicles were fully mature, as monitored by sonographical and endocrinological evaluation, human chorionic gonadotrophins (Profasi or Pregnyl) were then prescribed. The supplementation of the luteal phase was started on the day of oocyte pick-up and consisted of either human chorionic gonadotrophins (Pregnyl) or natural progesterone (Utrogestan or Proluton). The details of the ovarian stimulation and luteal-phase support protocols have been described previously.[3]

Semen evaluation and preparation

The semen analysis was carried out according to the recommendations of the World Health Organization.[4] A semen sample was considered to be normal when the following criteria were fulfilled : (i) sperm density 20 million/ml^3, (ii) progressive motility 50%, and (iii) at least 30% of spermatozoa with normal morphology. Sperm evaluation and preparation was done at least once prior to the treatment cycle in order to evaluate whether enough spermatozoa were present in the ejaculate to perform ICSI.

The semen preparation consisted of

centrifugation on a 90-70-50 Percoll discontinuous gradient without any other specific treatment.[5]

Oocyte preparation

Oocyte retrieval was performed by vaginal ultrasound-guided aspiration of the follicles, 34-38 hours after hCG injection. The oocyte-cumulus complexes were kept in the CO_2 incubator for 2-4 hours before being denuded. The cumulus cells were removed by incubation for 30-60 seconds in HEPES-buffered human tubal fluid (HTF) medium with 80 IU hyaluronidase/ml (type VIII, 320 IU/ml, Sigma Chemical Co., USA). The removal of the remaining cumulus and corona cells were then enhanced by aspiration technique. Afterwards, the oocytes were rinsed several times in HEPES-HTF medium and then assessed for their maturation ; i.e., germinal vesicle or metaphase-I or metaphase-II stage. Mature oocytes were grouped and then incubated in HTF medium at 37°C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2. About 2-4 hours later, the oocytes were assessed again to see whether more oocytes had become metaphase-II. ICSI was carried out only on the mature oocytes.

Intracytoplasmic sperm injection

The gametes were handled with Narishige micromanipulators under 200 x magnification using a Diaphot Nikon inverted microscope equipped with Nomarski differential interference contrast optics and fitted with a warming stage to avoid cooling of the gametes during micromanipulation (Fig. 1). The micropipettes for microinjection were made of glass capillary tubes with an outer diameter (OD) of 1.0 mm and an inner diameter (ID) of 0.5 mm. The micropipette was pulled on a micropipette puller (Narishige) and the opening was ground to the required size using a micro-grinder (Narishige). For microinjection needles, the 45° bevelled-tip micropipette with OD = 10 mm and ID = 5-7 mm was required. The oocyte holding pipettes were hand-pulled under Bunsen burner flame until an opening of OD = 100-150 mm and ID = 20-30 mm was obtained. They were then polished by the heated filament of a microforge. The holding and injection pipettes were connected to the syringe system which was filled with mineral oil.

Just prior to the injection, the sperm suspension was mixed in the ratio of 1 : 5 with 10% polyvinylpyrrolidone (PVP, MW 360,000, Sigma) in HEPES-HTF medium to facilitate handling and to prevent the sperm cells from sticking to the microinjection pipette during the procedure. A 25 ml-drop of the sperm suspension was then placed on a glass microinjection slide (Monash system)[6] close to the egg-containing drop (25 ml) of HEPES-HTF medium without oil covering. Only one oocyte was injected at a time so that it would stay outside the incubator for less than two minutes.

For the ICSI procedure,[2,7] a motile spermatozoon had to be immobilized by placing the injection pipette on the midpiece of the sperm tail and moving it sharply across the tail. The immobilized spermatozoon was then drawn tail-first into the injection pipette. The injection pipette was then moved to the egg-containing drop where an ovum was held steady by a suction-controlled holding pipette such that the polar body was in the 6 or 12 o'clock position. The injection pipette was quickly inserted, at the 3 or 9 o'clock position, through the zona pellucida and the egg membrane until the tip of the pipette was almost at the opposite side of the egg (Fig. 2). To assure that cyto-

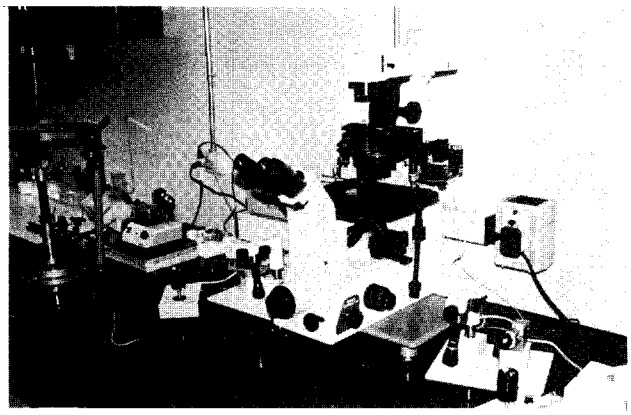

Fig. 1. A complete set of micromanipulator at Chiang Mai Ram Hospital, including Nikon Diaphot-300 inverted microscope fully equipped with Narishige micromanipulator and microinjector.

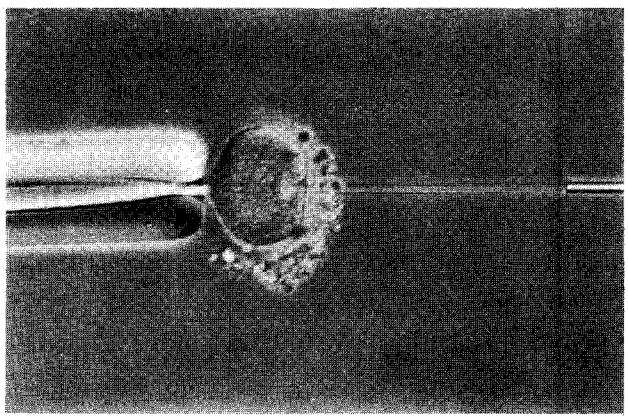

Fig. 2. ICSI procedure, showing the micro-injection pipette containing a single spermatozoon being pushed through the zona pellucida and the oolemma deeply into the ooplasm at 3 o'clock position while the oocyte being stabilized by the holding pipette.

plasmic placement did occur, a small amount of egg cytoplasm was pulled into the injection pipette. The cytoplasm together with the sperm were injected into the egg and the pipette was withdrawn quickly from the egg. The egg was released from the holding pipette and the pipette was withdrawn from the drop. The injected egg was then removed from the injection drop and was transferred to HEPES-HTF medium. The microinjected oocyte was then washed two to three times in HEPES-HTF medium and incubated in HTF medium at 37°C in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. The process was repeated until all the eggs had been injected.

Assessment of fertilization and cleavage

About 16-18 hours after the ICSI, the oocytes were examined under the inverted microscope for any sign of damage or fertilization. Fertilization was considered normal when two clearly distinct pronuclei containing nucleoli were present. The embryo cleavage of the two pronuclear oocytes was evaluated after a further 24 hours of in vitro culture. Cleaved embryos with less than 50% fragmentation were eligible for transfer. About 44-48 hours after the ICSI, up to three embryos were loaded into a few microlitres of HTF medium and into a Pivet catheter (Cooke, Australia) and transferred into the uterine cavity.

Establishment of pregnancy

Pregnancy was diagnosed by the increasing serum hCG concentrations on at least two occasions between 15 and 20 days post-embryo transfer. Clinical pregnancy was determined by observing the gestational sac(s) by means of sonography at 6-7 weeks of gestation. Prenatal genetic diagnosis was carried out by amniocentesis at 16 weeks of gestation. Prospective follow up study of the children born after ICSI was also planned.

Table 1. Overall data from 12 consecutive cycles of ICSI

No. of cycles	12
No. of oocytes collected	100
No. of injected oocytes (mature eggs)	88
No. of intact oocytes (post-ICSI)	70 (80%)
No. of degenerated oocytes (post-ICSI)	18 (20%)
No. of cycles with fertilization	12 (100%)
No. of fertilized oocytes (2PN)	43 (61%)
No. of cleaved embryos	38 (88%)
No. of embryos transferred	30 (79%)
Average no. of embryos per transfer	2.6 ± 0.5
Pregnancies (% per transfer)	3 (25%)
No. of sacs (implantation rate)	3 (10%)
Abortion (after 6 weeks)	2
Delivery (at 36 weeks)	1

Results

As shown in Table 1, a total of 100 oocytes were recovered in 12 cycles. Of these, 88 were microinjected and 70 (80%) remained intact. Sixty-one percent of those survived oocytes (43/ 70) fertilized normally (two pronuclei, two polar bodies) and the majority of them (38/43, 88%) cleaved. All patients received an embryo transfer of one to three "good-looking" embryos (average of 2.6 embryos per transfer) and three of them became pregnant (25% per embryo transfer). Among the pregnancies, there were two miscarriages and one ongoing pregnancy, giving the implantation rate of 10% (3 sacs/30 embryos). While the abortions occurred at 6 and 7 weeks of gestation, the ongoing pregnancy ended with a male baby of 36-week gestational age borned to a 28 year-old mother who, at that time, had developed pregnancy-induced hypertension. Her pregnancy was then decided to be terminated and the labour was successfully induced. The pregnancy finally ended up with the vaginal delivery of a healthy male 2,350 gm on the 7 th October 1995.

Overall, 12 of the 100 oocytes were not injected. This was due mainly to maturity status of the oocytes ; i.e., four of them were at germinal vesicle (GV) stage and the remaining had not yet extruded the first polar body (metaphase-I stage). All of those incompletely mature oocytes were not injected and were then excluded from this study.

Discussion

Assisted fertilization by the micromanipulation of gametes has been a revolutionary advance in the management of those couples who cannot achieve satisfactory fertilization

with conventional IVF.[1,2,7] In the past, subzonal sperm injection (SUZI)[8] and partial zonal dissection (PZD)[9] had been offered for those infertile couples with limited success. Since the first success of ICSI,[10] more and more extensive studies of this innovative technique have been reported with increasing successful results. Particularly with the Belgium experience on more than 3000 injected eggs, very high fertilization and implantation rates have been achieved from ICSI.[7] Since then ICSI has become the only established procedure of choice for assisted fertilization.

In this preliminary report, we have presented the protocol and the results of our first 12 consecutive ICSI cycles for patients with severe and largely intractable male factor infertility. The main indications for ICSI in our study were (i) too few (less than 1 million) motile spermatozoa harvested from the semen, and (ii) failure of fertilization in one, two or even more IVF treatment cycles.[11] Concerning the results of our ICSI programme, quite a few of the injected oocytes had been damaged, particularly in the very beginning of our experience, so that the percentage of degenerated oocytes was quite high compared to those of other reports. However, the fertilization rate (61%) and pregnancy rate per embryo transfer (25%) after ICSI were all comparable to those of routine IVF. Our results, therefore, confirm the results of a preliminary study by Palermo et al[10] and were quite consistent with results from the Belgium group.[2,7] To our best knowledge, this is also the first report of successful ICSI in Thailand.

Our ICSI technique is quite different to that of other "well-established" centres in that we have used glass slides for microinjection instead of plastic plate. This is due to the fact that Nomarski differential interference contrast optics of our system, which gives a very clear plane for the microinjection, has to be worked with a glass slide only. The other differences are about the pipettes, which are absolutely straight without any bending at the tip ; and oil-free drops of sperm- and egg-containing medium being used for ICSI. In that regard, the advantages are two-fold ; (i)less time-consuming for the pipette preparation, and (ii) avoiding the toxicity, if present, in some batches of mineral oil. In addition, since only one egg is injected at a time, the egg will stay outside the incubator for less than two minutes. Therefore, development of the injected eggs should theoretically be better than those staying outside for a longer period of time.

On the other hand, we absolutely agree with the others on some critical points of ICSI.[12] Firstly, it is important that the motile spermatozoon be completely immobilized before the injection. Secondly, the oocyte membranes have to be broken so that the spermatozoon has obviously been placed inside the oocyte cytoplasm. When these two procedures are implemented together with a strong background in micromanipulative techniques and a successful IVF programme, the results of ICSI should be satisfactory.

In conclusion, eventhough the number of treatment cycles in our study was too small for a final conclusion to be drawn at this moment, it seems that ICSI will play a major role in the treatment of male factor infertility. With ICSI, the use of donor semen would become obsolete and it would be able to offer most couples with the so-called "male factor infertility" excellent opportunities to have their own genetically derived children.

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REPRODUCTIVE SCIENCE

Varicocele in Men with Infertility Evaluated by Scrotal Physical Examination and Scrotal Ultrasonography : Its Correlation with the Sperm Count

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ABSTRACT

Objective To investigate 1) the correlation of scrotal physical examination comparing to scrotal ultrasonography in the detection of varicocele in men with infertility, depicting scrotal ultrasonography as the gold standard, 2) the clinical significance of ultrasound diagnosed varicocele by comparing incidence of oligospermia in patients with and without varicocele.

Design A prospective study.

Setting University Hospital.

Subjects A total of 110 men with infertility more than 1 year duration who were referred to the infertility clinic for the investigation and treatment of infertility.

Main outcome measures Sensitivity, specificity, positive predictive value, negative predictive value of scrotal physical examination in the detection of varicocele in men with infertility depicting scrotal ultrasonography as the gold standard and incidence of oligospermia in patients with and without varicocele.

Results The scrotal physical examination in the detection of clinical varicocele has sensitivity of 73.90%, specificity of 90.60% and positive predictive value of 85.00% with negative predictive value of 82.80%. Of total 110 patients, 64 had normal ultrasound scans. Twenty-two of 64 (34.38%) were found to have oligospermia. Among 46 patients with varicoceles diagnosed by ultrasonography, 26 (52.56%) were found to have oligospermia. Significantly, more patients with varicoceles were found to have oligospermia (P = 0.034).

Conclusion Physical examination of the scrotum which is a noninvasive,inexpensive, and convenient technique remains an appropriate screening test with reasonable accuracy but ultrasound examination should be used in doubtful cases especially those with difficulty to detect this condition by scrotal physical examination or those in whom we suspect subclinical varicoceles. Significantly more patients with varicocele were found to have oligospermia than those without varicocele.

Key words : varicocele in infertile men, scrotal physical examination, scrotal ultrasonography, oligospermia

Varicocele is an abnormal tortuosity and dilatation of the veins of the pampiniform plexus within the spermatic cord. It is approximately found in 30-40% of men seeking clinical evaluation for infertility.[1-3] The relationship between varicocele and male subfertility has been recognized since the late 19th century.[4] It has a potential of reducing all seminal parameters[5] which can be improved by surgical varicocele repair.[3,6] Given the association between varicocele and male subfertility, as well as the potential for enhanced fertility following varicocele repair, considerable attention has been devoted to improving techniques for the diagnosis of this lesion. The most common method of identifying varicocele is physical examination. This technique is convenient, inexpensive and noninvasive. Physical examination, however, is somewhat subjective and is dependent on the experience of the examining physician. Additionally, it has suggested that small varicoceles not detectable by physical examination alone (subclinical varicoceles) may have a role in subfertility and merit correction.[7,8] Therefore, physicians have used various diagnostic techniques other than physical examination to find these small varicoceles.

Adjunctive tests that have been used for diagnosis include venography,[9] radioisotope scanning,[10] thermography,[11] and Doppler ultrasound.[12] All these methods have significant disadvantages, however, and none is used universally. Real-time sonography is a possible alternative technique for diagnosis with the advantage of direct visualization of dilated vessels. Given suitable scanning equipment, the examination is fairly simple to perform, quick, and noninvasive. Since scrotal sonography has proved invaluable to detect many intrascrotal abnormalities and has the unique ability to visualize the testicle and surrounding structure,[13-15] we have decided to investigate 1) the correlation of scrotal physical examination comparing to scrotal ultrasonography in the detection of varicocele in men with infertility, depicting scrotal ultrasonography as the gold standard, 2) the clinical significance of ultrasound diagnosed varicocele by comparing incidence of oligospermia in patients with and without varicocele.

Materials and Methods

From September 1989 to March 1991, a total of 110 men with infertility more than 1 year duration were referred to our infertility clinic at The Royal Free Hospital for the investigation and treatment of infertility. The age range of the patients was 19-41 years (mean age 29.5 years). All patients underwent both scrotal examination and scrotal ultrasound. Scrotal ultrasound was

performed immediately after the scrotal physical examination. Scrotal examination was performed with the patient in the standing position before and during Valsalva's maneuver. Patients were assessed for the presence of varicocele as well as testicular size and consistency. All varicoceles identified at physical examination were classified as grade 1 (palpable only during Valsalva's maneuver), grade 2 (palpable without Valsava's maneuver), grade 3 (visible without the need for palpation).[16]

The instrument used for scrotal ultrasound was a high-resolution real-time scanner (Diasonics) with a frequency of 7.5 MHz. Examination was performed on both sides at rest and during Valsalva's maneuver in both supine and upright positions. Testicular length, width and anterior-posterior dimension were measured. The number of veins and the maximum diameter of the largest vein were evaluated.

In the supine position, the scrotum was supported by a towel wrapped around the upper thighs. Imaging was performed by direct contact of the transducer with the scrotal skin. Because the scrotal contents were easily deformed by the transducer, copious amounts of acoustic gel were used to facilitate scanning. The examiner scanned the scrotum by supporting the testicle with one hand while holding the transducer with the other hand. The vessels were scanned on each side from the hilum of the testicle to the scrotal neck. This was accomplished by rotating the testicle slightly in its long axis to bring the vessels to the lateral aspect of the scrotum and directly beneath the face of the transducer. Imaging through the testicle was thus avoided and proximity of the vessels to the transducer increased. Scanning then was performed with the patient upright and the examiner kneeling in front of the patient.

All patients were required to stand for 2 minutes before beginning scanning in the upright position to increase hydrostatic pressure within the veins. A varicocele was considered to be present by scanning if 2 or more veins could be identified, with at least 1 vein having a diameter of 3 mm. or greater.[7] All the patients had 2 semen analysis performed from the initial visit spaced at least 1 week apart. Semen samples were evaluated in accordance with WHO standards. A subject was considered to have oligospermia if he had a sperm count of less than 20 milliom/ml. All data are expressed as percentage. Statistical comparisons were expressed in term of sensitivity, specificity, positive predictive value, negative predictive value, and chi-square test.

Results

Of 110 men with infertility, clinically palpable varicocele was found in 40 patients (36.36%). Fifteen were of grade 1, nineteen of grade 2 and four of grade 3. Of these 40 patients, 32 (80%) had left-sided varicocele, 2 (5%) had right-sided varicocele and the remaining 6 patients (15%) had bilateral varicoceles. Of the 40 patients with clinically palpable varicoceles, 34 (85%) had the diagnosis confirmed by scrotal ultrasound. Of these 34 patients who had varicocele diagnosed by scrotal ultrasound, 17 (50%) had varicocele on the left side, 1 (2.94%) had varicocele on the right side and the remaining 16 patients (47.07%) had bilateral varicoceles. (Table 1) Seventy patients in whom initial scrotal physical examination failed to detect varicocele, 12 (17.14%) were found to have varicocele by scrotal ultrasound, all of which were on the left side. This group of patients was classified as subclinical varicocele. Statistical analysis revealed that scrotal physical examination in the detection of clinical varicocele has sensitivity of 73.90%, specificity

Table 1. The outcome of scrotal physical examination (SPE) and scrotal ultrasonography in the detection of varicocele

	clinical diagnosis by SPE	ultrasound diagnosis
left-sided	32 (80.00%)	17 (50.00%)
right-sided	2 (5.00%)	1 (2.94%)
bilateral	6 (15.00%)	16 (47.06%)
Total	40	34 (85.00%)

Table 2. Correlation between scrotal physical examination (SPE) and scrotal ultrasonography (U/S) in the detection of varicocele

		Scrotal U/S		Total
		Positive	Negative	
SPE	Positive	34	6	40
	Negative	12	58	70
Total		46	64	110

Sensitivity	73.90%
Specificity	90.60%
Positive predictive value	85.00%
Negative predictive value	82.80%

Table 3. Comparison between ultrasound diagnosed varicocele and oligospermia

	Varicocele diagnosed by U/S		Total
	Positive	Negative	
Patient with oligospermia	26	22	48
Patient without oligospermia	20	42	62
Total	46	64	110

Chi-square test = 4.775

P = 0.0344

of 90.60% and positive predictive value of 85.00% with negative predictive value of 82.80%. (Table 2)

Of total 110 patients, 46 had normal ultrasound scans, 22 of 64 (34.38%) were found to have oligospermia. Fourty-six patients who had ultrasound diagnosed varicoceles 26 (52.56%) were found to have oligospermia. Significantly more patients with varicoceles were found to have oligospermia (P = 0.034, Table 3). Of the 46 patients with varicoceles, 30 had bilateral varicoceles. Half of these patients had oligospermia. In the remaining 16 patients who had unilateral varicocele 11 (68.75%) were found to have oligospermia. More patients with bilateral varicoceles were found to have oligospermia than those with unilateral varicocele but the difference was not statistically significant (P = 0.363).

Discussion

Varicocele has been implicated as a cause of male infertility resulting from abnormal spermatogenesis. Various mechanisms have been suggested to account for this testicular dysfunction which included 1) inhibition of the thermoregulatory system of the pampiniform plexus with a noted increase in scrotal temperature[17,18] 2) peritesticular blood stasis leading to anoxic tissue destruction and/or impairment of epididymal function.[18] In this study the incidence of varicocele is 36.36%. Other studies showed the incidence of 30-40% in men seeking clinical evaluation for infertility.[1-3] Detection of this condition in the clinical practice is important as it is commonly found in men seeking clinical evaluation for infertility and also it is the most common surgically corrected cause of male infertility. Improvement in seminal parameters is demonstrated in approximately 50-70% of patients following surgical varicocele repair. Conception rate after successful operation is 40-50%[1,3,6]

The usual standard method of detection is scrotal physical examination. Although it is convenient, noninvasive and inexpensive its disadvantage is possible false positive and false negative results and consequently other techniques have been tried such as venography,[9] radioisotope scanning,[10] thermography,[11] and Doppler ultrasound.[12] All of these have their significant disadvantages and limitations.

At present ultrasonography is playing an increasing role in the clinical practice especially high-resolution model which has high ability to visualize the small structure therefore it can provide an alternative to other tests to detect this condition.[7,19]

From this study using scrotal ultrasound as the gold standard, of 70 patients whose scrotal physical examinations were normal 12 had varicocele detected by ultrasound (false negative 17.14%). All of them had left-sided varicocele. On the other hand, of the 40 patients diagnosed to have varicocele by scrotal physical examination 34 had diagnosis confirmed by ultrasound (false positive 15%). Bilateral varicoceles were found by ultrasound in 10 patients whom only unilateral varicocele was detected by scrotal physical examination. Therefore, clinical palpable unilateral varicocele may have a risk of misdiagnosis of bilateral varicoceles. Statistical analysis revealed that scrotal physical examination in the detection of clinical varicocele has sensitivity of 73.90%, specificity of 90.60% and positive predictive value of 85.00% with negative predictive value of 82.80%. We conclude that scrotal physical examination which is a nonivasive, inexpensive, and convenient technique remain an appropriate screening test with reasonable accuracy but ultrasound examination should be used in doubtful

cases especially those with difficulty to detect this condition by scrotal physical examination such as subjects with active reflexes, thickened spermatic cords, tight scrotums or those in whom we suspect of subclinical varicoceles. Recent study by Meacham and colleagues[20] attempting to find the incidence of varicoceles in the general population evaluated by physical examination, gray scale sonography and colour Doppler sonography found colour Doppler sonography to be more sensitive than physical examination and gray scale sonography as colour Doppler sonography has the theoretical advantage of allowing direct demonstration of reversed flow in the testicular vein. Colour Doppler sonography may identify a group of patients as having varicoceles who have a normal physical examination and negative gray scale sonographic evaluation. The only disadvantage of colour Doppler sonography is the extremely high cost of the machine which makes it not feasible to be available like ultrasound machine in most general clinical practice.

This study shows that finding of oligospermia increases significantly in men with infertility who were found to have varicocele comparing to those without varicocele. Reduction of semen quality was apparent in approximately 50 % of the men with varicoceles.[1,4] Similar to other studies, our results show 56.52% (26 of 46) of patients with varicoceles had oligospermia. Our findings and others indicate that many varicoceles are not associated with abnormal semen analysis. We suggest that not all varicoceles necessitate surgical correction. The presence of detectable varicocele associated with abnormal semen analysis in an infertile couple should be an appropriate indication for treatment especially varicocele repair after the female partner has been completely evaluated.

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REPRODUCTIVE SCIENCE

Investigation of Maternal Immunologic Reaction to the Paternal Alloantigens in Couples with Habitual Abortions of Unknown Aetiology

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ABSTRACT

Objective To study maternal immunologic reaction to the partner's alloantigens in one-way mixed lymphocyte culture.

Design Cross-sectional study.

Setting Department of Obstetrics and Gynaecology, University of Belgrade and Kragujevac, Yugoslavia.

Subjects The study group consisted of 9 couples with three or more successive spontaneous abortions without liveborn children and without known endogenous and exogenous causes of habitual abortions. The first control group comprised of 9 couples with liveborn children, while in the second one there were randomly selected 9 couples without children.

Main outcome measures Immunologic status including functional capacity of lymphocytes, T-lymphocytes response to division-disabled foreign histoincompatibility antigens of partner's lymphocytes, T-lymphocytes response to partner's alloantigens.

Results Women from study and control groups had no statistically significant differences in general immunologic status (functional capacity of lymphocytes). Women in the study group, T-lymphocytes response to stimulation by disabled partner's lymphocytes was significantly lower compared to both control groups ($P < 0.05$). However, immunologic reaction of women in study group to alloantigens of men from control groups did not show any significant difference compared to control groups results ($P > 0.05$).

Conclusion In couples with habitual abortion of unknown aetiology immunologic factors have a very important role.

Key words : habitual abortion, paternal alloantigens, immunoregulation

In human population one part of the genetic material fetus inherits from mother, and the other one from father. So, conceptus is always allogenic to the mother. In pregnancies with haemochorial type of placenta exist a direct contact between fetus and mother, and from immunologic point of view fetus has all features of allograft.[1] Medawar was the first who noticed that pregnancy represents a distinct immunologic paradox.[2] He investigated why there's no immunologic rejection of fetus in pregnancy. There are many theories explaining the mechanism that prevents immunologic rejection of fetus, but there is no an appropriate answer. Today, there are numerous evidences that maternal reaction to fetus is the usual event in normal pregnancy with features of immunologic enhancement of both humoral and cellular mechanisms.[3-5] Habitual abortions (HA) appear in 0.5% of all couples. The great number of them are of unknown aetiology. The aim of this study was to evaluate the influence of immunologic factors on habitual abortions of unknown aetiology.

Materials and Methods

During 1995 we investigated 22 couples with three or more consecutive spontaneous abortions without liveborn children. By diagnostic methods we eliminated presently known endogenous and exogenous causes of HA in thirteen couples. Nine remaining couples with unexplained aetiologic factors of HA comprised our experimental group. Control group A included women and their partners with liveborn children (not more than two), and in control group B were randomly selected women and men without children. After test of blast transformation of lymphocytes as a screening of general immunologic status (functional capacity of lymphocytes) for all women from experimental and both control groups, we performed one-way mixed lymphocyte culture (MLC) test in order to investigate immunologic reaction of women. We investigated their T-lymphocytes respond to foreign histocompatibility antigens of partner's lymphocytes disabled for division (stimulator cells). Immunologic reaction in experimental group of women to stimulator cells of partners from control groups was also studied. Human peripheral blood mononuclear cells were obtained by Ficoll-Hypaque separation of heparinized venous blood. After three washes, they were resuspended in the culture medium, autoclavable RPMI-1640 medium supplemented with 2 mM glutamine, 100 U/ml penicillin, 10 um/ml streptomycin, 10 mM HEPES, 0.34% NaHCO3 and 2% fetal calf serum (FCS) (Serva, Feinbiochemica). The procedure resulted in 3 ml of final suspension with $1x10^6$ cells/ml. All stimulator cells were treated with mitomycin C (100 μg/ml, Sigma), to prevent DNA synthesis and to keep cells viable at the same time. Then, cells were washed three times and resuspended in the medium. Stimulator cells and responding cells of couple were mixed and in triplicate placed in 0.2 ml volume into the flat-bottomed wells of microtiter tissue culture plates (Limbro). The cultures were put in a 37°C incubator containing 5% CO_2, for 4 days, pulsed with 1 μCi of 3H-Thymidine (Amersham-life science) and harvested 18-24 hr later. These

culture conditions were found to be optimal in preliminary experiments. The filters were air-dried overnight and then placed in vials with Toluene and Liquiflour (New England Nuclear). The amount of incorporated 3H-Thymidine was determined on beta-counter (LKB-Wallac-1219 Rackbeta), and found to be proportional to the intensity of division, i.e. DNA synthesis in responding T-lymphocytes. Obtained data were interpreted as the mean of counts per minute (CPMmean) incorporated by identical triplicate cultures, and tested by using a one-way analysis of variance.

Results

Prevalence of habitual abortions of unknown aetiology, i.e. idiopathic HA were presented in Fig. 1.

Immunologic reaction of women i.e. their T-lymphocytes respond to division-disabled foreign histocompatibility antigens of partner's lymphocytes (stimulator cells) in one-way MLC was presented in Table 1 and Fig. 2.

Values of CPMmean did not show any significant shift from mean value in all nine experiments of each group separately. There were statistically significant differences in study group (couples) 1, 2, 4, 6, 7 and 9. ($P < 0.05$)

In 6 out of 9 couples in study group, response of women's T-lymphocytes to partner's alloantigens was statistically lower, compared

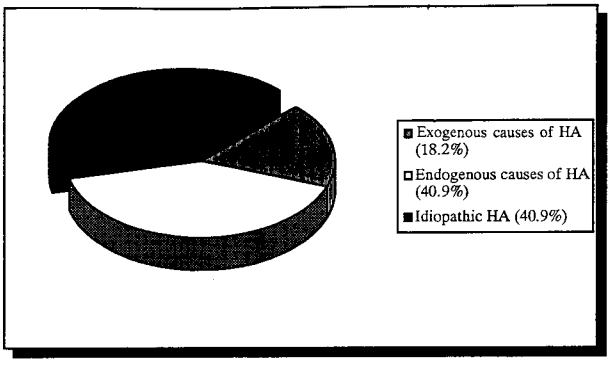
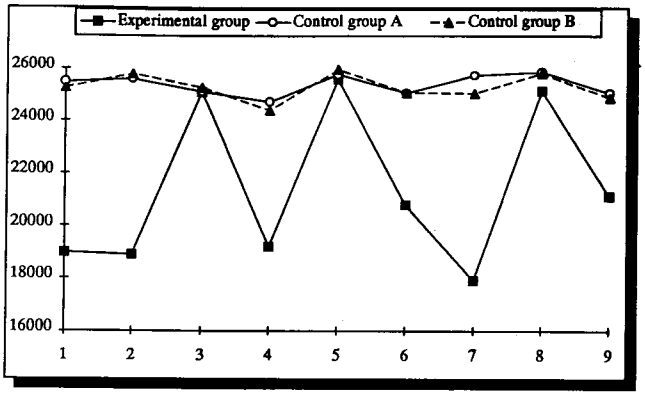


Fig. 1. Aetiologic factors of HA.

Table 1. Immunologic reaction of women to alloantigens of partner in MLC

Experiment No :	Study group (CPMmin)	Control group A (CPMmin)	Control group B (CPMmin)
1	18,974.9	25,468.2	25,243.7
2	18,890.4	25,591.3	25,788.4
3	25,094.7	25,100.3	25,267.1
4	19,181.1	24,708.6	24,379.4
5	25,577.2	25,769.2	25,966.2
6	20,775.5	25,053.0	25,059.3
7	17,885.5	25,722.6	25,036.1
8	25,131.4	25,857.9	25,800.8
9	21,111.5	25,049.0	24,873.1
Xmin	21,402.47	25,368.9	25,268.23
SD	2,885.8	379.19	481.19
CV	13.48	1.49	1.9



CPMmin (1) : Women from study group - partners from controls A

CPMmin (2) : Women from study group - partners from controls B

Fig. 2. One-way MLC in study and both of control groups.

to both control groups. ($P < 0.05$) (Table 2).

Immunologic reaction of women from study group, where their T-lymphocytes responded to division-disabled foreign histocompatibility antigens of partner's lymphocytes from control groups are represented in Table 3 and Fig. 3.

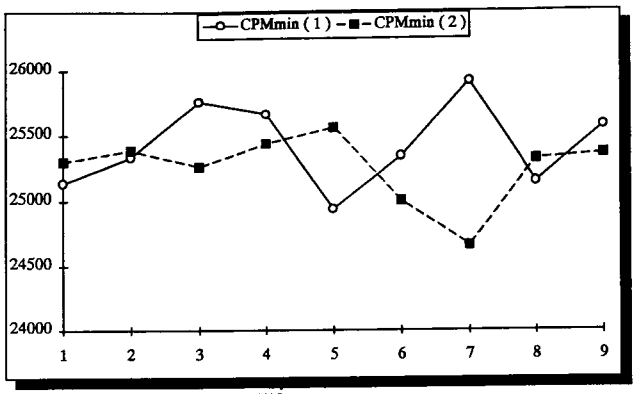
There were no significant shift from mean value in all nine experiments. Comparison of CPMmean values between newly formed groups and control groups did not show significant differences in all nine experiments ($P > 0.05$), (Table 4).

Table 2. Differences of CPMmean (one-way MLC) in investigated women

Experiment No :	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Control group A and B / Study group	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p < 0.05$

Table 3. Immunologic reaction of women from study group to alloantigens from controls (A and B)

Experiment No :	**CPMmin (1)**	**CPMmin (2)**
1	25,138.1	25,301.2
2	25,335.4	25,387.7
3	25,758.2	25,261.4
4	25,661.7	25,434.6
5	24,937.3	25,554.9
6	25,338.3	25,005.2
7	25,911.8	24,659.5
8	25,141.8	25,315.3
9	25,565.5	25,355.5
Xmin	25,420.9	25,252.8
SD	305.4	252.26
CV	1.2	1



CPMmin (1) : Women from study group - partners from controls A

CPMmin (2) : Women from study group - partners from controls B

Fig. 3 Women T-lymphocytes response to alloantigens of partners in controls.

Discussion

Working on experimental animals, Beer et al found that immunologic reaction of mother to fetus is usual event in normal pregnancy and it represents prerequisite for establishment and function of immuno-regulatory mechanisms that prevent immunologic rejection of fetus in pregnancy.[6,7] They found no blocking antibodies in sera of 30% women with HA. They believed that this antibodies have a key role in the prevention of immunologic rejection of fetus. Blocking antibodies paradoxically seem to develop or to be enhanced by paternal antigens.[8]

Lack of effectors of immunologic reaction can be a cause or consequence of lowered immunologic response that we have in our

Table 4. Statistical significance of differences of CPM mean (one-way MLC) in women from control groups and women from newly formed groups

Experiment No :	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Control group A and B / Women from Study group, partners from control group A	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Control group A and B / Women from study group, partners from control group B	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

investigations in one-way MLC. This finding is probably induced by partner's similar HLA genotype.

In analysis of HLA system in couples with HA of unknown aetiology, Thomas et al confirmed significantly more frequent A-locus, B-locus and D/DR-locus, than in couples with normal pregnancies.[9] They believed that division of HLA alleles can cause division of recessive lethal genes bound to HLA locus. Faulk and co-workers suggested that recognition of trophoblast lymphocyte cross-reactive (TLX) antigens by mother's immuno-system is necessary.[10] Greater histocompatibility of these antigens leads to immunologic rejection of placenta and recurrent spontaneous abortions. Our investigation confirmed that HA of unknown aetiology (i.e. idiopathic HA) comprises 40.9% of all HA. Size of response of T-lymphocytes from women in study group to stimulation by division-disabled lymphocytes of their partners (in one-way MLC) is significantly lower in 6 out of 9 cases, compared to both of controls ($P < 0.05$). However, immunologic reaction of women from study group to alloantigens of partners from control groups didn't show significant differences to control groups results ($P > 0.05$). In women from study group and from both of control groups, there were no statistically significant difference in general immunologic status (functional capacity of lymphocytes). According to our study it can be concluded that immunologic factors have an important role in a great proportion of habitual abortions with unknown aetiology.

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CASE REPORT

Monosomy 22 : A Case Report

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ABSTRACT

A new case of monosomy 22 is described in a female newborn. The child had dysmorphic face, bilateral corneal opacity, elongated, disproportional toes and muscular hypotony, and systolic heart murmur was detected. Chromosome analysis identified karyotype 45, XX, -22, inv (9). The newborn died shortly after birth. Postmortem examination showed persistent truncus arteriosus type III, atrial septal defect and persistent left superior vena cava. This is the fifth case of monosomy 22 which is incompatible with life.

Key words : Monosomy 22

Monosomies of autosomal chromosomes in human generally lead to early embryo death. However, several cases of liveborn with monosomy of G group chromosomes have been reported, which were always associated with multiple congenital malformations. Medline Search of the literature showed that up to December 1994 there were four cases of monosomy 22 : two without mosaicism[1,2] and two in the mosaic form.[3, 4]

Here we report a new case of monosomy 22 in a liveborn child.

Case Report

A female proband was born at term after an uncomplicated pregnancy and syntocinon induced delivery. The 24-year-old mother and a 33-year-old father were healthy, as well as their first child, a 3-year-old girl. There was no consanguinity, no history of miscarriage, and no family history of birth defects.

Birthweight of the proband was 2,950 gm (percentile 10-25 for gestational age (GA)), length 46 cm (below the percentile 3 for GA), head circumference 34.5 cm (percentile 10 for GA). She had slanting palpebral fissures, bilateral corneal opacification, flat nasal ridge, low-set and poorly formed, soft ears and short neck. The toes were elongated and disproportional. The genitalia was female, with prominent labia minora. Muscular hypotony was dominant. Shortly after birth, a systolic heart murmur was detected.

Screening echocardiography showed double output of right ventricle (DORV) and atrial septal defect (ASD).

Blood count examinations showed thrombocytopenia. Routine biochemical studies were consistent with respiratory acidosis. Microbiological evaluations were normal.

She died of cardiac failure on the 14th day after birth.

Autopsy findings : The heart defect was a persistent truncus arteriosus type III (TAP III). Atrial septal defect (ASD) in the region of fossa ovalis was noted. There was persistent left superior vena cava. The right lung had two lobes. Other autopsy findings were unremarkable.

Cytogenetic analysis : Chromosome analysis was performed on trypsin G-banded and Q-banded chromosome preparations from peripheral lymphocyte cultures ; 100 metaphases were scored. In all cells, a 45, xx, -22, inv(9) (p12q13) karyotype was present (Fig. 1). The pericentric inversion was inherited from the mother, whose karyotype was 46, xx, inv (9). The father had normal male karyotype. A study of the chromosome 22 short arm polymorphism indicated that the proband's single chromosome 22 had maternal origin (data was not shown).

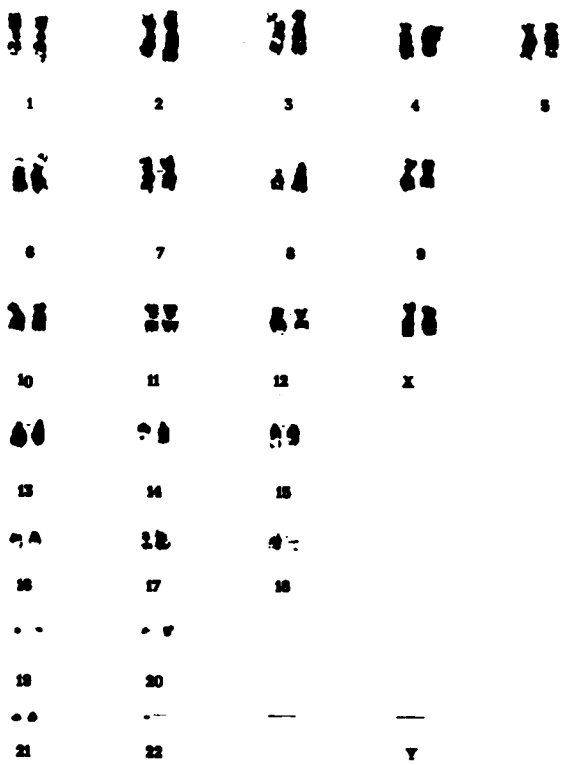

Fig. 1. Karyotype 45, XX, -22, inv(9), tripsin-Giemsa banding. (magnification 100x)

Discussion

Monosomy 22 is an extremely rare chromosomal abnormality. Up to now there were only two cases without mosaicism have been reported.[1,2] The cited probands died shortly

Table 1. Cases of monosomy 22 with or without mosaicism

	Rosenthal et al	De Cicco et al	Moghe et al	Verloes et al	Our case
Karyotype	45, XY, -22	45, XY, -22	46, XY/45, XY, -22	46, XY/45, XY, -22	45, XY, -22, inv(9)
Facial dismorphy	+	+	+	+	+
Eye anomalies	+	-	-	-	bilateral CO
Microcephaly	-	+	+	+	-
Anomalies of extremities	"lobster claw" appearance of the hands and feet	minor	cutaneous syndactyly between all the fingers	minor	minor
Cardiovascular anomalies	PDA	POF, bilat.VCSA	-	-	TAP III. pers.LSVC,ASD
Muscular hypotonia	-	-	+	+	+
Others	agenesis of the thymus	agenesis of the olfactory bulbs and tracts, 5 accessory spleens	moderate PMR, short stature	moderate PMR, short stature	right lung consisted of two lobes

Abbreviations : CO = corneal opacity ; PDA = patent ductus arteriosus ; POF = pentalogy of Fallot ; bilat.VCSA = bilateral vena cava superior arteries ; TAP III = persistent truncus arteriosus type III ; pers.LSVC = persistent left superior vena cava ; ASD = atrial septal defect ; PMR = psychomotor retardation

after birth. Moghue et al[3] and Verloes et al[4] reported cases of mosaic monosomy 22 with 24% and 9.4% monosomic cells respectively. Although we carried out chromosome study of a single tissue, the clinical and cytogenetic features of our patient seem to be consistent with true monosomy 22 (Table 1).

The patient described by Rosenthal et al had agenesis of the thymus and PDA, anomalies consistent with DiGeorge syndrome (DGS).[1] DGS is a developmental defect of the 3rd and 4th pharyngeal pouches, resulting in maldevelopment of the great vessels, thymus and parathyroids. DGS is heterogenous with genetic and nongenetic causes.[5] Several chromosome abnormalities may lead to this syndrome.[6] The association of DGS and partial monosomy 22 was proved for the first time by de La Chappele et al.[7] The region critical for DGS lies within 22q11.[6,8,9] The patient with monosomy 22 described by De Cicco et al,[2] and our own patient had unusual cardiovascular anomalies which are common in DGS.[10] De Cicco et al did not note signs of cellular immune deficiency and hypoparathyroidism, nor the absence of the thymus and parathyroids on autopsy. Our patient had normal serum calcium level without hypocalcemic seizures. Postmortem examination for the presence of parathyroid tissue was not requested. The thymus gland proper was present.

Future cases of monosomy 22 would be of great value in order to delineate the clinical syndrome associated with this chromosome aberration.

The very commonly found pericentric

inversion of chromosome 9 should be regarded as a normal variant. Some investigators suggested significant relationship between the chromosome 9 pericentromeric heterochromatin polymorphism and nondisjunction of the acrocentric chromosomes.[11] Our proband did not meet these features as it had single chromosome 22 and pericentric inversion of chromosome 9 both inherited from the same parent.

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CASE REPORT

Wandering Splenomegaly, the Cause of Pelvic Mass : A Case Report

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ABSTRACT

A married hill-tribe woman aged 35 years was presented with a left asymptomatic pelvic mass for 7-8 months. The operative findings revealed a large wandering spleen of normal general appearance and the other normal abdomino-pelvic organs. She was treated by splenectomy. No complications were found postoperatively and within 3 months of follow up. Wandering splenomegaly is a rare cause of pelvic mass, but the gynaecologist should be beware of its possibility.

Key words : wandering splenomegaly, pelvic mass

Pelvic mass is an usual gynaecological condition.[1] The mass commonly originates from the reproductive organs, e.g., the uterus, oviducts and ovaries, but bladder, caecum, colon and lymph nodes may be the origins. The pathologic processes of mass include inflammation, infection and neoplasm. The enlarged spleen descending to be pelvic mass is a rare condition. This case report presents a married hill-tribe woman who came to Fang Hospital with a pelvic mass of 7-8 months duration and was diagnosed to be wandering splenomegaly postoperatively.

Case Report

A hill-tribe female farmer, 35 years of age, came to Fang Hospital with a complaint of asymptomatic mass at left lower abdomen for 7-8

months and no abnormal symptoms concerning defecation and micturition. On history taking, she had come to a community hospital 3 months ago because of this mass and the operation was performed. After the operation, the mass still appeared and she was not told of what had been done or what was the nature of the mass. Continued growing of the mass caused her to come to Fang Hospital. Her menstrual cycles were regular. She could not remember her last menstrual period and contraception was not used. She had two children who died of fever at one and two years of age respectively.

On physical examination, the general appearance of patient was good with no fever, no anaemia and no jaundice. The vital signs were namely ; temperature 36.8°C, pulse rate 80/min, blood pressure 110/70 mmHg and respiratory rate18/min. All systems were examined and found to be normal, except a low midline scar and a mass in the left pelvis. The mass had firm consistency and was 15 x 20 cm in size, smooth surface, freely mobile in all directions, not tender and could be palpated clearly apart from the uterus. The provisional diagnosis was left ovarian tumour.

Pre-operative laboratory investigations were haematocrit 42 % volume, white blood count 5,400 cell/cu.mm., neutrophils 64 %, lymphocytes 33 % and eosinophils 3%, adequate platelets, blood group "O" and normal chest film.

The operation was performed under general anaesthesia. A low midline incision on the previous scar was made from just below the umbilicus to the symphysis pubis. The findings revealed a small amount of clear colourless peritoneal fluid, normal uterus, both oviducts and ovaries (Fig. 1). The liver and other abdominal organs except spleen, were normal. The abnormal mass was located in the left pelvic cavity and lied on the reproductive organs. The external feature of that abnormal mass appeared like a spleen. It was deep red, smooth surface, firm consistency, and 20 x 12 x 3 cm in size. (Fig. 2,3) It also had 20 cm stalk which ended near the stomach. The mass was removed together with clear colourless peritoneal fluid for cytology. The operating time was 55 minutes. Blood loss was minimal and no immediate complications occurred during operation.

The patient had routine postoperative care without antibiotic. The blood was drawn for malarial detection, haemoglobin typing and red blood cell morphology and all of which resulted in negative findings for malarial detection, AA_2 Hb typing and normal red blood cell morphology. Aspiration of bone marrow revealed normal findings. No complications occurred.

The patient was discharged on the fourth postoperative day, but she did not go home until the tenth postoperative day waiting for her cousin to take her home.

One week later, she came back to the hospital with a fever. Malarial infection was diagnosed and she was admitted. Plasmodium vivax infection was treated by chloroquine and primaquine for three days. She was discharged on the fourth day after admission and was told to take further 12 day course primaquine at home.

On follow up at one and three months interval after operation, the patient was found to be normal on examination.

Pathological findings : (Fig. 4,5,6)

Peritoneal fluid : no malignant cells are detected.

Spleen :

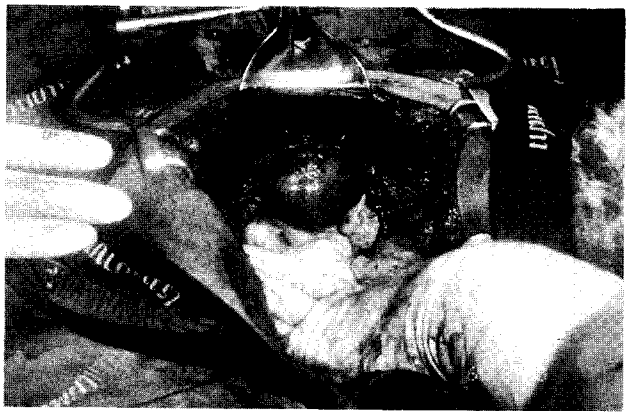


Fig. 1. Normal pelvic organs.

Fig. 2. Pelvic mass-wandering spleen.

Fig. 3. Pelvic mass-wandering spleen.

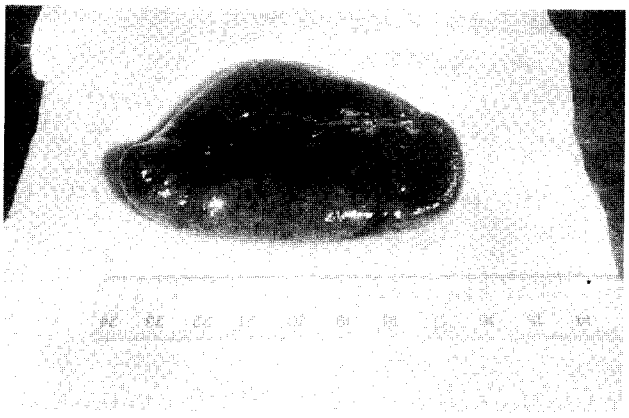
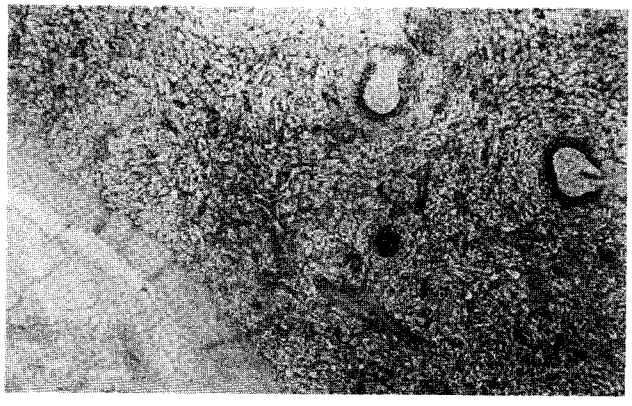


Fig. 4. Macroscopic appearance of the spleen (20 x 12 x 3 cm).



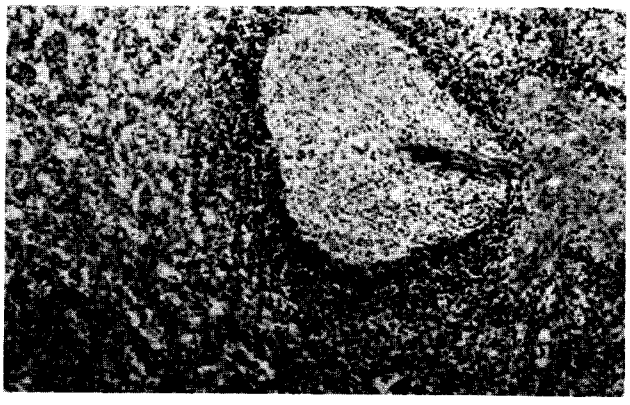


Fig. 5, 6. Microscopic description : enlarged white pulps with lymphoid follicles and prominent germinal centres. There are congested red pulps and extramedullary haemopoiesis.

Macroscopic description : specimen measures 20 x 12 x 3 cm. Multiple sections present prominent white pulps and congested red pulps.

Microscopic description : enlarged white pulps with lymphoid follicles and prominent germinal centres. There are congested red pulps and extramedullary haemopoiesis.

Diagnosis : Spleen, hyperplastic white pulps and reticuloendothelial hyperplasia with extramedullary haemopoiesis.

Discussion

Ectopic spleen, otherwise known as "wandering spleen" or "floating spleen", is a very rare condition.[2-5] From the collection of 97 reported cases in the world,[2] Abell concluded that most cases were caused by congenital or acquired stretching and lengthening of the anchoring peritoneal folds, suspensory ligaments or abdominal muscles. These combined conditions made the spleen to be malposition.[2,3] Abell also found that 92 of these 97 cases were female.[2] Ectopic spleen might be asymptomatic or symptomatic. In the case of symptomatic ectopic spleen, the patient usually presented with intermittent and/or chronic abdominal or pelvic pain, nausea, vomiting, or melena. The pain was from intestinal obstruction or distension or torsion of the pedicle and infarction of the spleen, resulting in rupture. The most serious complication of ectopic spleen was rupture of spleen.

Barloon reported a case that presented with a very large abdominal mass and no other abnormal symptoms.[6] Operative and subsequent pathologic findings showed an ectopic spleen infiltrated by a well-differentiated lymphoma. Nino Murcia et al reported a case of 75-year old woman suffering from chronic lymphocytic leukemia and presented with frequency of micturition.[7] On physical examination revealed a pelvic mass, which was further investigated by ultrasonography and computed tomography. The investigation demonstrated a large ectopic spleen in the pelvis causing pressure effect on the bladder. Furthermore, ectopic spleen may result from splenomegaly caused by chronic malaria[8,9] or other unexplained caused of splenomegaly.[2,4,5]

Preoperative diagnosis of ectopic spleen is unusual because it is so rare that makes the surgeons ignore it. However, in the suspected case, there are many investigations that can be done preoperatively, e.g., ultrasonography, computed tomography, etc.[7,10] The suggestive treatment of ectopic spleen is splenectomy in all of symptomatic cases because of its serious complication as mentioned above.[2,3,9] In this case, the cause of the ectopic spleen may be originated by a congenital condition that caused lengthening of the anchoring peritoneal folds and suspensory ligaments and followed by relaxation of abdominal muscles from pregnancy and parturition. The final precipitating cause is thought to be splenomegaly from chronic malaria. Although, history of chronic malarial infection cannot be derived, the patient's residency in the endemic area of malaria and her return to hospital one week postoperative for treatment of malarial infection are the possible reasons. Splenectomy was performed in this case to prevent the mentioned serious complication.

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REVIEW

Oocyte Cryopreservation

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From a practical standpoint, oocytes can be donated by volunteers, from patients undergoing laparoscopic sterilization, and women who are willing to donate their excess oocytes after IVF programme.[1] A major difficulty in a donation programme consists in the synchrony between the ovarian cycles of donor and recipient. If this synchrony is not present at the time of donation, the oocytes can, if possible, be cryopreserved and then thawed at an appropriate time in the course of a subsequent cycle. The availability of an adequate oocyte cryopreservation programme will increase the chance of establishing a donor programme.

Furthermore, in some countries it might be more acceptable on ethical grounds to freeze-thaw oocytes than embryos. It also would be beneficial to young women at risk who had lost ovarian function due to chemotherapy, surgery, or certain pelvic diseases. This would be analogous to sperm banks for men who are at risk to lose their reproductive function ; when techniques of oocyte freezing become reliable, egg banks could offer similar alternatives.[2]

All of the techniques used in cryopreservation of embryos have been studied on oocytes of various animals[3] such as mouse, rat,[4] hamster,[5] pig[6] and cattle,[7] with wide-range of success. Extensive studies, however, have been done only in mouse oocytes, expanding from the most immature primary follicles,[8] germinal vesicle stage,[9] up to matured ovulating oocytes,[9] with increasing orders of post-thaw survival rates, even though the results are much lower than those of embryo cryopreservation.[10]

Biology of mature oocyte

Structural and biochemical aspects of both oocyte growth and maturation have been fully reviewed by Wassarman.[11] Therefore only those aspects relevant to cryopreservation will be outlined in this review. In addition to its extraordinary large size, the oocyte also contains structures which are sensitive to cooling and warming. Those include cytoskeletal elements, meiotic spindle, oolemma, zona pellucida, and cortical granules.[12]

Microtubules and microfilaments are involved in many cellular processes such as cell division, cell motility, control of cell shape and cytoplasmic organization. These processes are of great importance for the development of an organism, and the organization of cytoskeletal elements in the mammalian egg is crucial not only during oocyte maturation but also for the events following fertilization, such as resumption of meiosis and polar body formation, migration of the pronuclei and formation of the first mitotic spindle. Oocytes arrested at metaphase-II contain a peripheral, anastral spindle apparatus, with microtubules extending from each pole to the kinetochores of bivalent chromosomes. Chromosomes at the metaphase plate and pericentriolar material associated with each pole act as microtubule organizing centres for tubulin polymerization during spindle formation. During metaphase II, Golgi-derived cortical granules are positioned immediately subjacent to the oolemma where they are triggered to undergo exocytosis at fertilization. Release of cortical granule contents consequently alters the zona pellucida (cortical granule reaction), creating a block to polyspermy. The oolemma of the mammalian oocyte is covered with numerous microvilli containing an actin filament core. Very little is known about the biochemical composition of the bilayer of this membrane ; however, one might speculate that the oolemma is easily destabilized, as it is poised for fusion with the fertilizing sperm and cortical granules.[12]

Problems associated with oocyte cryopreservation

Since the organelles which are necessary for fertilization of frozen-thawed oocytes and further development of the embryo could be affected by cryoprotectant(s) and /or process of cryopreservation, a number of problems arise. These mainly include decreased rates of normal fertilization and increased rates of chromosomal abnormalities.[12,13]

One of the proposed mechanisms which explains the decrease in rates of fertilization in dimethyl-sulphoxide(DMSO)-exposed oocytes at 37°C, both of the mouse[14,15] and human,[16,17] is zona pellucida hardening. Use of fetal bovine serum to protect against zona hardening induced by DMSO has also been reported recently.[18] Furthermore the freezing/thawing process itself also affects the zona pellucida of mouse oocytes and consequently reduces the rate of fertilization. The nature of this effect, however, is still unclear, whether premature cortical granule release or a direct effect of freezing/thawing itself.[19] Propanediol(PROH) at high dose (>1.5 M) can also induce profound changes in the organization of the cytoskeleton and has detrimental effects on mouse oocytes.[20] Parthenogenetic activation of mouse oocytes by PROH has also been reported.[21] A dramatic increase in polyspermy has been observed after cryopreserving human oocytes in either PROH and sucrose (40% of fertilized oocytes) or DMSO (20% of fertilized oocytes).[22]

Disruption of microfilaments in mouse oocytes by DMSO, at 37°C, has been reported[23] and is considered to be a cause of the increased frequency of polyploid embryos derived from frozen-thawed oocytes. From chromosome spreads, more digynic than diandric chromosomal patterns were identified. This would suggest that the failure of oocytes to extrude their second polar bodies is a likely cause of polyploidy rather than polyspermic fertilization.[24,25] Moreover, there is concern about an increased risk of aneuploidy in em-

bryos that develop from mouse ova exposed to cryoprotectants (DMSO, PROH)[26] and to freezing-thawing.[27] Since the preovulatory oocyte is arrested in metaphase of the second meiotic division, its chromosomes are still attached to microtubular spindles. Because of the sensitivity of these spindles to temperature changes, it is feared that cryopreservation could cause their depolymerization, which would interfere with the separation of sister chromatids at fertilization. This would result in chromosome nondisjunction and aneuploidy after extrusion of the second polar body. An increase in aneuploidy would increase the incidence of miscarriage and may increase birth defects.

In meiosis, spindle development follows the breakdown of the germinal vesicle. Therefore, it could be postulated that freezing of immature mouse ova, prior to germinal vesicle breakdown, might avoid injury to the spindle. Extensive studies, including electron microscopic examination of germinal-vesicle(GV)-stage mouse oocytes after vitrification, have been conducted by van Blerkom.[28] Following vitrification in vitrification solution-I(VSI), 83% (410/495) of mouse GV-oocytes were capable of resuming meiosis and undergoing chromosomal and cytoplasmic maturation to the metaphase-II stage. However, the results clearly demonstrated that vitrification was associated with chromosomal and cellular disorders that could adversely affect development after fertilization. These irreversible changes included (i) premature chromosomal condensation, (ii) mixing of the nucleoplasmic and cytoplasmic components prior to GV breakdown, and (iii) externalization of chromatin fragments into the cytoplasm after reformation of the oocyte nucleus, which shows the potential for the generation of fertilizable oocytes containing deleted segments of DNA. These would consequently produce chromosomal abnormalities in the offspring.[28]

Progress in oocyte cryopreservation

Even though oocytes are especially sensitive to cryopreservation procedures, progress has been made in oocyte cryopreservation primarily by adapting protocols used successfully with preimplantation mammalian embryos. Early reports have been reviewed elsewhere.[29-31] The following review will show some of the results involving slow and rapid cooling of both mature and immature mammalian oocytes.

In earlier reports, in vivo matured mouse oocytes were frozen slowly using DMSO as the cryoprotectant to achieve complete dehydration, and normal offspring were obtained after in vitro fertilization. However, fertilization rates were significantly lower than for nonfrozen oocytes.[32] Studies on mouse eggs indicated that DMSO should be added at 0 °C and the eggs slow cooled to temperatures of -80 °C or lower before storage in liquid nitrogen.[33] This result has also been substantiated by Todorow et al.[34] Using a combination of PROH and DMSO as the cryoprotectant, they reported higher morphological survival rates, fertilization rates and developmental rates of frozen-thawed mouse and hamster oocytes when cooled to a low (-80 to -110 °C) than to a high intermediary temperature (-35 °C). Recently, George et al obtained satisfactory results from cryopreservation of mouse oocytes.[35] They slowly froze and thawed mature mouse oocytes in 1.5 M DMSO at +4°C. After thawing, the incidence of fertilization did not differ from that in control group of oocytes, and after fertilization, the ability of the fertilized frozen-thawed oocytes to develop to the blastocyst stage in vitro was only

slightly less (77%) than that of the controls (87 and 89%). As regards the cryoprotectants, it has recently been shown that PROH was superior to glycerol and DMSO in allowing development of frozen/thawed mouse oocytes to 2-cell embryos.[36,37] It has also been shown that in vitro matured mouse oocytes undergo normal development up to the blastocyst stage at rates not different from nonfrozen controls, using the freezing procedure with DMSO as the cryoprotectant and slow cooling to -80°C.[38]

Other than the mouse, few other animals have been tested for the feasibility of oocyte cryopreservation using slow cooling methods. For instance, hamster oocytes equilibrated in 1.5 M PROH were cooled slowly to -75 °C before being plunged into liquid nitrogen. Of 1340 frozen-thawed oocytes, 94% survived and were equivalent to fresh oocytes in their capacity for sperm penetration.[39] Studies in the rabbit showed that 9% of post-thawed oocytes developed to live young following in vitro fertilization and embryo transfer, compared to 32% of those in a control group. In cattle, Lim reported that only 17.6% and 0.4% of frozen-thawed oocytes, equilibrated with glycerol, cleaved and developed to blastocyst respectively.[40,41]

Using a modified vitrification solution (90% VSI) from Rall and Fahy[42], Nakagata[43] obtained very high success rates for cryopreservation of murine oocytes. Of total 348 oocytes cooled rapidly, approximately 88% were morphologically normal after thawing and 78% developed to 2-cells, 46% of which, following embryo transfer, resulted in normal live births. In another report,[44] however, only 54% of mouse oocytes vitrified by the Nakagata method survived morphologically. Recently, a comparable success rate of oocyte cryopreservation has been reported. Following in-vitro fertilization of vitrified (VSI) mouse oocytes which were diluted with a glycerol/sucrose solution, up to 51% of transferred embryos (25% of vitrified oocytes) developed to live young.[45]

Using the ultrarapid freezing method (3.5M DMSO + 0.5M sucrose), Surrey and Quinn reported comparable rates for fertilization and development to blastocyst stage, for frozen-thawed mouse oocytes when compared to those obtained after freezing by the slow cooling method.[46] For hamster oocytes, Lewin et al also reported a viability rate of 82.3% and a sperm penetration rate of 27% following cooling rapidly with 3M DMSO + 0.25M sucrose.[5]

Cryopreservation of immature (GV) oocytes is challenging in the sense that, if feasible, it can provide a means of preserving oocytes before maturation and avoid the possible damage that may occur in the spindle of mature oocyte during freezing and thawing. All the studies that have been done on this issue, however, are still preliminarily and mostly limited to murine oocytes. Mandelbaum et al found that morphologic survival of immature mouse oocytes after slow cooling was only half that of mature ova.[47] Schroeder et al also demonstrated that the development of frozen-thawed mouse GV-oocytes were severely impaired compared to that of metaphase-II-oocytes.[9] On the contrary, Candy et al recently reported a very promising results on cryopreservation of immature mouse oocytes.[48] Using DMSO (1.5 M) as the cryoprotectant, they slowly froze germinal vesicle (GV)-stage mouse oocytes down to -40 °C before plunging it into liquid nitrogen and thawed rapidly in air for 40 seconds and then in 30-35 °C water until the ice melted. The overall survival rate of those frozen-thawed GV-oocytes was 27% which

compared favourably with the estimated overall survival of mature oocytes cryopreserved by a similar procedure. For the rat, it was shown that immature rat oocytes could be successfully cryopreserved when they were surrounded by five or more layers of cumulus cells.[4] So far, oocytes from farm animals have not been frozen successfully with subsequent in vitro fertilization and the production of normal offspring.[31] In vitro maturation of bovine immature oocytes was significantly reduced after cooling and very few oocytes (6%) survived freezing.[7] In contrast to other mammalian oocytes, GV pig oocytes are very sensitive to cooling. Oocyte death occurred when oocytes were cooled to 15 °C or lower.[6] Very interestingly, Carroll[49] and Gosden et al[50] have respectively been able to restore fertility to oophorectomized mouse and sheep by transplantation of frozen-thawed primordial follicles (ovarian autographs). This innovative procedure might be practicable and extrapolatable to human use in the near future.

Human studies

Most of the studies on human oocyte cryopreservation have used conventional slow freezing methods to either high or low intermediary temperature.[30] To date, only five pregnancies have been reported following IVF of cryopreserved human ova using slow cooling in DMSO.[22,51,52] Even though up to 80% of morphologically intact human oocytes can be recovered after rapid freezing and thawing, their fertilizability and developmental ability have not been substantially confirmed.[19] In the past, the rates of development of thawed ova in vitro are still very low when compared to those obtained with embryos. For example, a summary of data in the USA showed that only 6.3% (8/127) of frozen-thawed oocytes survived, fertilized and were considered worth transferring to patients and no pregnancies were obtained.[53] However, as more and more experiences have been gained, the results of human egg cryopreservation is improving. For example, Toth et al recently demonstrated that immature (prophase-I) human oocytes from stimulated IVF cycles were able to survive cryopreservation and resume meiosis to achieve full nuclear maturation post-thaw.[54] In addition, these cryopreserved oocytes also retained the same capacity for fertilization and development as control nonfrozen oocytes. Moreover, Gook et al recently reported an encouraging result from cryopreservation of mature human eggs using propanediol (PROH) procedure.[55] Their result showed that the rates of normal and abnormal fertilization of these survived (50%) frozen-thawed oocytes were comparable to those of normal IVF. Normal ultra-structures within the cell including chromosomal arrangement were also demonstrated in all of these cryopreserved oocytes undergoing normal fertilization.

Even though variations in experimental detail are numerous, a survey of results provide several overall impressions. First, the preferred cryoprotectants for freezing oocytes are DMSO and PROH for conventional protocols and ultrarapid freezing while vitrification solutions are generally modifications of those of Rall and Fahy. Second, oocytes maintain their morphological integrity under a variety of freeze/thaw regimens and encouraging fertilization and cleavage rates have been reported. However, when results from the literature are evaluated on the basis of viable offspring/oocyte frozen, it is clear that only a small percentage of frozen oocytes can be fertilized and develop to term in most species regardless of the developmental

stage or method used. Third, better results are possible with mouse oocytes, for which success rates of 15-30% viable offspring/oocyte frozen have been obtained with both conventional protocols and vitrification.[12] Fourth, due to the above-mentioned deleterious effects of both the cryoprotectants and the process of freezing-thaw itself, attempts to find intrinsically less toxic cryoprotectants and new techniques for rapid cooling and vitrification of cells have occurred. Fifth, antifreeze glycoproteins (AFGPs), from Antarctic nototheniid fishes, which have been shown to have cryoprotective properties, were found to facilitate and significantly enhance the survival of pig oocytes cooled rapidly by the new technique called "directional solidification".[56] Sixth, obviously, the ability of the AFGPs to protect cells during cryopreservation could have numerous applications in the near future.

Conclusion

In view of legal and ethical considerations involved in embryo cryopreservation, the desirability of oocyte preservation is widely accepted.[53] Although a small number of human unfertilized mature oocytes have been cryopreserved using various methods, success rates are still very low. Methods for the cryopreservation of eggs should be developed, but these methods probably should be proved by animal experiments to be safe, especially with regard to genetic damage, before a policy of transfer of embryos derived from frozen-thawed human ova is applied on a large scale.

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COMMENTARIES

Ethics and Reproduction "Primum non nocere"

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The **raison d'etre** for medicine is to do good and not to harm our patients. This noble objective is important and needs to be reminded time and time again. The reasons for this reminder are several.

First, in Thailand, society has changed over past decades. The nation has developed tremendously over the last 35 years, under the national socio-economic planning. The 7th plan will end this year. All these successive national plans emphasized "Economic development" using people as **resources** for development and not development to better human conditions. Only the 8th up coming plan emphasized **people** as the centre of development. And yet after seven successive plans, development is still not sustainable. There are islands of poverty amidst the sea of plenty. Environmental degradations is rife. Conflicts of interest are being fought over natural resources, such as forest lands, water resources, and other natural finite resources. Big money politics rules over the just and equitable politics. No wonder then life and living conditions for the majority of our nation has not progressed for better, even though the economic indicators indicated otherwise.[1] The gap, between the haves and havenots, continue to widen further.

Second, Medicine reflects society. The development of medicine emphasized technological equipments.With "Hi-tech" came "Lo-touch". Most medical school and private hospitals compete to obtain the most advanced technologic equipment available. It has been reported that Bangkok has more CT scanners per million population than Australia, Switzerland, Sweden, France and United Kingdom, although the health and the economy of her people lag way behind those developed countries.[2] With the emphasis of economic development in the forefront, professional ethics take a back seat. Commercialism takes the front seat. Medical profession is not the only profession under scrutiny, almost all the professional bodies, engineering[3,4] mass media,[5] even religious bodies[6] came under criticism.

Medicine differs from other professions in that it has compassion as its core. Medicine is not commerce. It is not for profit. Deviation from this principle is the root cause of the erosion

of public trust and respect.[7] If Thai medicine is to follow the "Hi - tech" and "market place" pattern of the U.S we can expect the same route of spiraling cost of health care, more frequent litigations, more defensive medicine, higher cost of health care[8] and further erosion of public trust.

It has been said that the more complicated and technological advanced the medicine, the easier the professional ethics are forgotten.[7] The more technological advanced and commercialised we become, the easier the exploitation of our patients.[9,10]

Our professional ethics were born over a few thousands years ago to prevent the profession from exploiting the less fortunate, the sick, the poor and the ignorants. The primary duty of physicians is to apply knowledge and skill to the best of our ability in a way that promotes the health and welfare of our patients.

Exploitation occurs because of several factors. Firstly, it occurs in situations where there is inadequate training, training in this case include professional ethic training. Each doctor may be well trained technologically but most of the time ethical training is taken for granted and therefore lacking. Secondly, new technological development "explodes" continually. These new technological developments occur so fast that the regulatory process lags far behind.[11-13] In fact these advances seem to occur in an ethical and regulatory vaccuum. Thirdly, exploitation occurs in commercial enterprise, that is enterprise with profit as its main motive.

No wonder exploitation occurs more readily in the milieu of modern reproductive medicine. The factors of inadequate ethical training combined with the explosion of technology of reproduction, and the outlook of reproductive medicine as a profit organization with market mechanism as a controlling factor further fuel the fire of exploitation. The mushrooming of private hospitals or fertility clinics with the physician or group of practicing physicians as the owner, selling high technology of reproduction, competing for limited number of patients has the ingredient for commercialism and hence exploitation. This is not only confined to Thailand.[9]

In commerce, the term **"Caveat Emptor" (beware buyers')** may be appropriate for consumers in prevention of exploitation by the merchants. Commercialism means marketing. Marketing means advertisement. In medicine advertisement, though prohibited by the profession, can take a subtle form of a news report of a new "scientific discovery". The new "scientific discovery", instead of being reported to the peer professional group through congress or in a professional scientific publication with a strict peer review, now takes the form of sensational reporting in a daily newspaper with high circulation.[14,15] Another form of subtlety is in a form of an interview in a glossy women's magazine. Again, this also is not only confined to Thailand.[16]

"Caveat Emptor", does not work in medicine. The reason for this is that for the reproductive health consumer to decide what is good for herself she must have correct, appropriate and timely information. Thai health consumers seeking reproductive health care do not have correct, adequate and timely information to make decision. A recent article in the Bangkok Post stated "Consumers abuse of technology normally occurs out of ignorance, but doctors abuses stem from handsome kick backs. Nevertheless, the worst case of technology abuse could be when a doctor assumes three roles in a hospital - as a major share holder, a manager, and a physician. When business's

survival is at stake and profits is the ultimate goal, abuses become even more likely."[17,18]

What then is the role of the Royal Thai College of Obstetricians and Gynaecologists (RTCOG) ? Education is the main objective of any professional college. Education to the public at large by the College will reduce the consumers abuse of technology. It will innoculate the public against exploitation by the profession.

Education to the specialist in training with emphasis on the professional ethics. Educating the specialist in training to understand truly the meaning of **"Noblese Oblige".**

Establishment of a "standard of care" by the College would outlaw fringe practice. Providing resources for second opinion would help the public to protect itself. Public sanction of the culprit would be the last resort.

It is not unreasonable for the public to expect the RTCOG to do all these to protect them and the profession.

If we do not keep our house in order perhaps some day someone else will do so.

"PRIMUM NON NOCERE"
(FIRST, DO NO HARM)
HIPPOCRATES
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