ISSN 0857-6084

THAI JOURNAL OF OBSTETRICS AND GYNAECOLOGY

THE OFFICIAL JOURNAL OF
THE ROYAL THAI COLLEGE OF OBSTETRICIANS
AND GYNAECOLOGISTS

VOL. 4 NO. 1 JANUARY - JUNE 1992

Thai Journal of Obstetrics and Gynaecology

ISSN 0857-6084. The Official Journal of the College of Obstetricians and Gynaecologists of Thailand.
Vol. 4 No. 1 January - June 1992

CONTENTS:

Effect of Pethidine on the Contractility of Myometrial Strips from Human Term Gravid Uterus in Vitro

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Abstract: *The effect of pethidine on the contractility of myometrial strips was studied in vitro. Myometrial strips were taken from the lower segment of 32 term parturients, undergoing low transverse caesarean section at Siriraj Hospital. Nine parturients were excluded from the study because their myometrial strips showed no spontaneous contraction in vitro. Of the 23 parturients with active strips, 10 were in latent phase and 13 were in active phase. The myometrial specimen from each parturient was divided into 2-6 small strips of equal size which were then randomly used as either control or experimental strips. The pattern of isotonic contraction was studied. In comparison with the time-matched control, pethidine in therapeutic concentrations (0.25, 1, and 4 μg/ml) did not exert any effect on the amplitude and period of myometrial contraction. However, at the extra-high concentration of 40 μg/ml, which was 160 times greater than the therapeutic serum level, pethidine lowered the amplitude or strength of myometrial contraction in the active phase group, while the period of contraction was shortened in both the active and latent phase groups. Further study is suggested to confirm these effects in vivo. (Thai J Obstet Gynaecol 1992; 4: 1-6.)*

Key words: pethidine, myometrial contraction, in vitro

Pethidine, a morphine derivative, has long been used as an obstetric analgesic. Its effect on the uterine contractility became a point of interest in 1943 when Gilbert and Dixon[1] observed that pethidine shortened the course of labour. Despite the fact that many investigators have reported the effect of pethidine on the uterine or the myometrial contractility, the results still remain inconclusive. The results may be enhancement, inhibition or no effect at all[2-11]. The controversy based on several different factors, such as study design, dosage of pethidine, route of drug administration, contamination with other agents, condition of the subjects, data and parameters analysis, etc.[12-13].

The objective of this study is

to investigate the direct effect of pethidine on the myometrial strips from the lower segment of human term gravid uterus, *in vitro*.

Materials and Methods

Informed consents were obtained from 32 term gravidas (37-42 weeks of gestation). Unfortunately 9 parturients were excluded afterwards because their myometrial stips showed no spontaneous contraction *in vitro*. Among the 23 parturients who fulfilled our experimental criteria, 10 were in latent phase and thirteen in active phase of labour. None had previously scarred uterus. None received any drugs potentially effecting uterine contractility within 24 hours before operation. These women underwent caesarean section under epidural anaesthesia in the Department of Obstetrics and Gynaecology at the Faculty of Medicine Siriraj Hospital, Mahidol University, from January to December 1989.

A small segment of uterus was excised from the upper margin of the uterine incision during low transverse caesarean delivery. The tissue was rapidly immersed in Kreb's solution[14], then delivered to the laboratory to be immediately tested or stored at 4° C over night and tested the next day.

In general, each uterine specimen was divided into 2-6 small strips, approximately 10x5x2 mm in size. These myometrial strips were dissected in such a way that the long axis of collagen fiber oriented parallel to the long axis of the strips. One end of each strip was anchored to a L-shaped glass rod in a 25 ml smooth muscle chamber containing Kreb's solution at 37° C, pH 7.35-7.45, continuously gassed with 95% O_2 and 5% CO_2, the other end was suspended to a displacement transducer (Myograph type A:NARCO Bio-system, part No. 705-0001) which transformed mechanical events to electrical impulses then transferred the impulses to a physiograph (NARCO Bio-system : amplifier type 7070, part No. 7160038). The experimental sets were left not more than 3 hours for the regular pattern of isotonic contraction to be detected. Any strips without spontaneous contraction within 3 hours were discarded. The active strips were randomly taken as either a control or experimental strip. In this study the control strip was called "time-matched control" because it was compared simultaneously with the experimental strip.

The baseline contractions were observed for 30 minutes then the interventions were performed. One hundred microliters of distilled water was added to the control chamber. At the same moment, 100 microliters of diluted pethidine was added to the experimental chamber. The contractions were observed for another 30 minutes then the tracing of myogram was obtained for analysis.

The tracings were sent to an assistant for data measurement to prevent observational bias. The parameters of contraction analysed in this

study were the amplitude and the period. A period of contraction was defined as the time - interval between the beginning of a contraction of interest and the beginning of a previous contraction[14]. A period of contraction represents a reverse assessment of frequency within a brief period of time. The contractility changes were presented in ratio between the value of parameters which averaged 30 minutes after the intervention (AF) to those before the intervention (BE), or AF/BE. The difference between AF/BE of the experimental strips and that of the control strips was assessed by Wilcoxon signed rank test. The *p value* of less than 0.05 was considered to represent a significant difference.

Results

The characters of the 23 parturients including age, length of gestation, gravidity, parity, number of abortion and indications for caesarean section are presented in Table 1. The pattern of contraction before the intervention was represented by the period averaged for 30 minutes before intervention as shown in Table 2. In the latent group, the average period of control strips was 10.70 ± 6.08 minutes and that of the experimental strips was 12.00 ± 7.78 minutes. In the active group, they were 11.92 ± 7.72 minutes and 11.75 ± 6.02 minutes respectively. The data, as tested by Wilcoxon signed rank test, demonstrated that the pattern of contraction before any intervention was the same in the control and in the experimental groups.

Table 1 Characters of the 23 parturients

Characters	Mean	SD
Age (yr)	24.30	3.98
GA (wk)	39.65	1.68
Gravida	1.52	0.93
Para	0.22	0.51
Abortion	0.35	0.36
Indications for C/S		n
Breech		18
Fetal distress		3
Placenta previa		2

Table 2 Patterns of the contraction before intervention

Groups	Period (minutes)			p
	n	Mean	SD	
Latent				0.1827
Control	24	10.70	6.08	
Experiment	24	12.00	7.78	
Active				0.3934
Control	35	11.92	7.27	
Experiment	35	11.57	6.02	

The effects of pethidine on the contractility of myometrial strips are shown in Table 3. In comparison with the time-matched control, therapeutic dose of pethidine, e.g. the concentration of 0.25, 1 and 4 μg/ml (22, 23), did not exert any effect on amplitude and period in the latent group. Similar results were obtained in the active group except at the dosage of 4 μg/ml

where the period was slightly decreased. However, at the extra-high concentration of 40 μg/ml, which was 160 times greater than the therapuetic serum level, pethidine lowered the amplitude or strength of myometrial contraction in the active phase group, while the period of contraction was shortened in both the active and latent phases groups.

Discussion

From this *in vitro* study, it was illustrated that pethidine in a therapuetic dosage did not exert any marked direct effect on both the period and amplitude of contraction of the myometrial strips from the lower uterine segment. The effects on the strips from latent and active phase parturients were similar. This finding contrasted to those of many recent studies, both *in vivo* and *in vitro*, which reported the enhancement effect trend[2-8].

It is difficult to interpret those

Table 3 Ratio of the parameters after intervention (AF) to the parameters before intervention (BE)

Group	Pethidine		AF/BE						
Active	dose		Control		Experiment		Difference		p
	(μg/ml)	n	Mean	SD	Mean	SD	Mean	SD	
Amplitude	0.25	11	1.01	0.15	1.01	0.20	0.00	0.26	0.4646
	1.00	9	1.00	0.17	1.11	0.29	0.11	0.35	0.1871
	4.00	7	0.93	0.13	0.92	0.27	-0.01	0.34	0.4329
	40.00	8	0.91	0.09	0.72	0.06	-0.20	0.14	0.0086
Period	0.25	11	1.08	0.41	1.23	0.40	0.15	0.66	0.1870
	1.00	9	1.00	0.13	1.17	0.46	0.17	0.45	0.3392
	4.00	7	1.06	0.10	0.94	0.12	-0.13	0.18	0.1455
	40.00	8	1.00	0.11	0.74	0.24	-0.27	0.31	0.0178
Group	**Pethidine**		**AF/BE**						
Latent	**dose**		**Control**		**Experiment**		**Difference**		**P**
	(μg/ml)	**n**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Amplitude	0.25	6	0.94	0.26	1.02	0.15	0.08	0.24	0.2315
	1.00	8	0.97	0.32	1.01	0.32	0.04	0.58	0.4443
	4.00	6	0.96	0.12	1.04	0.18	0.08	0.10	0.1727
	40.00	4	0.87	0.20	0.68	0.17	-0.19	0.24	0.0721
Period	0.25	6	1.12	0.45	1.08	0.26	-0.04	0.28	0.3766
	1.00	8	1.03	0.40	0.13	0.36	0.11	0.35	0.1313
	4.00	6	1.19	0.34	1.15	0.50	-0.04	0.46	0.3766
	40.00	4	1.08	0.36	0.61	0.08	-0.48	0.32	0.0339

in vivo studies because many conditions effecting uterine contractility may act as confounding factors. Example of these factors are gestational length, point of labour, status of membranes, drug dosage, and route of administration. Besides, research protocols such as condition of control and methods of result measurement and analyses have to be considered[12-13].

Among *in vitro* studies[9-10], this research possessed two major differences from others. They were the subjects (human vs. animal) and the concentrations of pethidine (therapuetic vs. very high level). The concentrations used in this study were more reasonable because these levels could be found in the serum of parturients receiving a therapuetic dose of pethidine[15].

Although considerable factors and environment can be controlled *in vitro*[16] and the accuracy of the results may be improved, these results can not be extrapolated to *in vivo*. Pethidine, *in vivo*, may indirectly effect the uterine contractility by its action on the neuro-endocrine system[3-5]. Thus, further *in vivo* studies are still necessary to confirm this *in vitro* finding. The appropriate study design, e.g. double-blind randomized control trial, is preferred to those of previous reports but the ethic is also a matter of concern.

From this *in vitro* study, pethidine in the therapuetic dosage did not exert any marked direct effect on either amplitude or period of contraction of myometrial strips from lower uterine segment. In extra-high concentration, pethidine reduced the strength of meometrial contraction but increased its frequency.

Acknowledgement

The authors would like to thank Assoc. Prof. Dr. Pachara Visutkul, Head of the Department of Physiology and Prof. Dr. Suporn Koetsawang, Head of the Department of Obstetrics and Gynaecology for their kindness in supplying the necessary materials used in this study, and to Prof. Dr. Sommai Toongsuwan for his valuable comments.

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Termination of 99 Dead Fetuses by Condom-Balloon Technique

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Abstract: *A total of 99 cases of dead fetus in utero in the second trimester of pregnancies, justified with indication for termination were prospectively recruited into a non-randomized clinical trial by using the condom-ballon technique with subsequent augmentation by oxytocin infusion. The patients were from 17-46 years of age with a mean of 27.19 years and duration of gestational age ranged from 13-32 weeks with a mean of 22.92 weeks. The success rate for expulsion within 24 and 48 hours was 78.8% and 94.95% respectively. The average of induction-expulsion (IE) time was 16 hours 36 minutes and not significantly different between primiparity and multiparity. The morbidity in this study was minimal.*

The results of this study indicate that the condom-balloon technique offers an appropriate modality management for dead fetus in utero because of high efficacy, minimal complications and inexpensive techinque. Further investigations to compare it with other technique should be undertaken. (Thai J Obstet Gynaecol 1992;4: 7-13.)

Key words: termination of pregnancy, condom-balloon technique, dead fetus in utero (DFU)

Dead fetus in utero (DFU) is a common problem in obstetric practice, especially in the second trimester, since subsequent various complications can occur[1,2], i.e. psychic problems, infections, consumptive coagulopathy and problems of fertility in the future. Most of these problems result from prolonged retention of fetus in utero and these problems can be reduced by early termination of pregnancy.

Various techniques of termination of DFU in the second trimester have different disadvantages[1-4]. Expectancy may be appropriate, but not too prolonged, because 80% will have spontaneous labour in two weeks after death[3]. The patient is usually anxious and desires to have a quick delivery. Technique of dilatation and curettage has definite risks of bleeding, uterine perforation, cervical tear, and incomplete abortion[4]. Hypertonic solution

technique may be complicated by hyperosmolar crisis, heart failure, septic shock, peritonitis, water intoxication, consumptive coagulopathy, and myometrium necrosis[1-3]. Prostaglandins is widely used now due to their high efficacy and acceptable side effects[5]. However, it may not be appropriate for routine use, since it is rather expensive.

The preliminary report of condom-ballooning technique, a simple new technique, indicates that it has high efficacy, minimal complications and is much cheaper[6]. At Maharaj Nakorn Chiang Mai Hospital, this technique is now routinely used for termination of pregnancy in second trimester. This study aimed to evaluate the efficacy of termination of DFU and its complications.

Materials and Methods

All of the patients were prospectively recruited from April 1984 to August 1991. The subjects were selected from patients with DFU during 12-32 weeks of gestation, without labour pain and with no contraindication for condom-balloon technique which included cervicitis, chorioamnionitis, rupture of membranes, undiagnosed fever, and leakage of amniotic fluid. The same technique were performed in all patients, and only by the authors.

Preparation of condom-balloon

The condom-balloon consists of 1) rubber urethral catheter #14-16, 2) condom, 3) silk # 3/0, and 4) catheter guide. The condom and catheter were sterilized with cidex solution for 15 minutes. The condom-balloon can be easily prepared by tying the sterile condom to a sterile catheter tip with 2 knots of silk to make a balloon at the tip of catheter. We defined the length from the tip of the condom to the knot 3.5 inches, and the length of catheter inserted in condom is 2 inches. The excessive portion of condom was excised. The preparation is illustrated in Figure 1.

Technique of insertion of condom-balloon

After grasping the anterior lip of the cervix by tenaculum, the empty condom-balloon was gradually inserted through the cervical os by using uterine forceps until the knot was slightly above the internal os. Usually, it can be easily inserted. If a catheter guide is used, it must not be inserted beyond the internal os. Then the balloon was inflated with normal saline. The amount of normal saline employed depends on uterine size, i.e. 50-100 ml, 100-150 ml, 150-200 ml, 200-250 ml, and 250-300 ml for 12-16, 16-19, 20-23, 24-27, and 28-32 weeks size respectively. After inflation of the balloon, the catheter was tied with silk, folded and hidden in the vagina.

Care after balloon insertion

Bed rest or normal activity

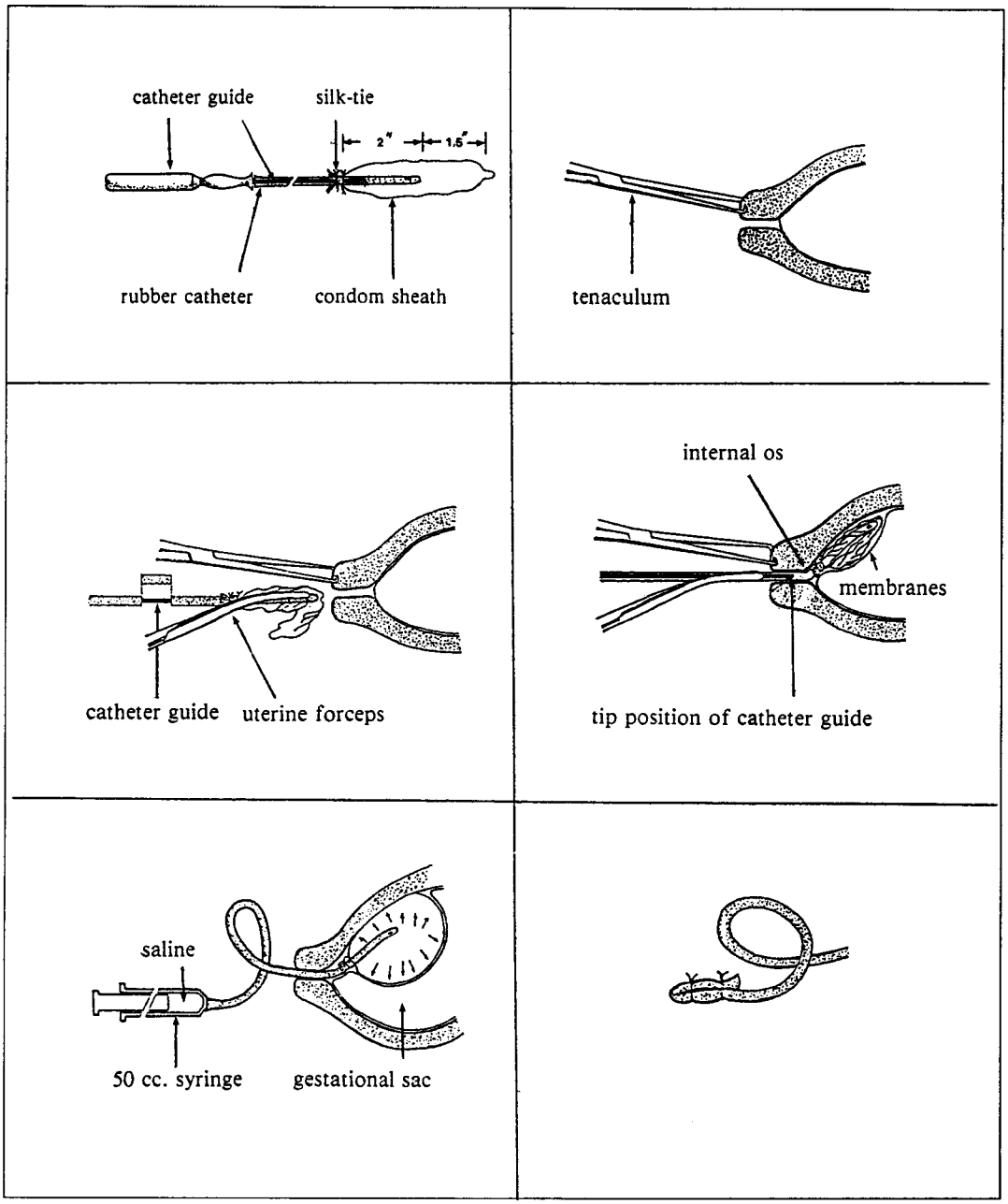

Fig. 1 Condom-balloon technique

was permitted. The patient was observed until spontaneous contraction was established, and then oxytocin was augmented to adjust for appropriate contraction. The balloon will be expulsed after cervical dilatation and must not be inserted back. The patient was attended until complete abortion. All steps and clinical changes were recorded for subsequent evaluation.

Results

Of 99 patients recruited, the average age was 27.19 ± 5.6 years, ranged 17-46 years, 50 were primiparae and 49 were multiparae. Distribution of patients' age and parities are shown in Tables 1 and 2. Average gestational age was 22.92 ± 5.0 weeks, ranged 13-32 weeks. Average uterine size was 21.51 ± 4.9 weeks, range 12-32 weeks. Distribution of gestational age and uterine size are shown in Tables 3 and 4 respectively.

Success rate for abortion (Table 5) within 24 and 48 hours occured in 78 (78.8%) and 94 (95.0%) cases, respectively. 5 patients (5.05%) failed to start contraction and aborted in the first 48 hours, one of them subsequently aborted in 72 hours and the four others were successfully terminated by sulprostone 48 hours after balloon insertion. Average induction-

Table 1 Distribution of age

Age	No. of patients	Percent
15-19	6	6.1
20-24	28	28.2
25-29	33	33.4
30-34	20	21.2
≥ 35	12	12.1
Total	99	100.0

Table 2 Distribution of parity

Parity	No. of patients	Percent
0	50	50.5
1	34	34.3
2	5	5.1
3	7	7.1
4	3	3.0
Total	99	100.0

Table 3 Distribution of gestatioal age

GA. (Weeks)	No. of patients	Percent
12-15	7	7.1
16-19	25	25.2
20-23	15	15.2
24-27	31	31.3
28-32	21	21.2
Total	99	100.0

Table 4 Distribution of uterine size

Size (Weeks)	No. of patients	Percent
12-15	9	9.1
16-19	27	27.3
20-23	24	24.2
24-27	24	24.2
28-32	15	15.2
Total	99	100.0

labour time (IL) was 9 hours and 23 minutes. (562.92 minutes) and average of induction-expulsion time (IE) was 16 hours and 36 minutes (996.15 minutes).

found in 5 patients, blood loss of more than 500 ml (associated with curettage) in 1 patient, febrile morbidity (>38.0° C) during retaining balloon in uterine cavity in 2 patients, and

Table 5 Number of patients for each induction-expulsion (IE) time period

Parity	IE < 24 hours	IE < 48 hours	IE > 48 hours	Total
0	37	46	4	50
1	32	34	0	34
2	4	5	1	6
3	4	6	0	6
4	1	3	0	3
Total	78	94	5	99

Average IL time between primiparae (9 hours 32 minutes) and multiparae (9 hours 14 minutes) was not significantly different ($p > 0.05$).

Average IE time between primiparae (16 hours 24 minutes) and multiparae (16 hours 48 minutes) was also not significantly different ($p > 0.05$). Of 94 patients whose terminations were successful with condom-balloon, 9 (9.6%) required further curettage due to incomplete expulsion. 29 of 94 (31%) aborted with intact membranes.

Significant complications were febrile morbidity after expulsion in 2 patients, however, there was no other evidence of infections among them. No serious complications occurred during the study.

15 (16%) of the patients had a minimal amount or almost no blood loss, 66 (70.2%) had a small amount of blood loss (<150 ml), 12 (12.8%) had moderate blood loss (150-500 ml) and only 1 had blood loss of more than 500 ml.

Table 6 shows the outcome of therapy.

Table 6 Outcome of therapy among 99 patients

Excellent (IE time < 24 hr, no curettage, no complication)	64.6 %
Good (IE time < 24 hr, curettage and/or minimal complications)	22.2 %
Fair (IE time) 24 hr but < 48 hr, or moderate complications)	8.1 %
Unsatisfactory (IE time > 48 hr, or serious complications)	5.1 %

Discussion

Condom-balloon technique has been used as a conventional method of second trimester termination at Maharaj Nakorn Chiang Mai Hospital for 15 years. From this study, the success rate of termination by this technique within 24 and 48 hours was 78.8 and 95.0% respectively and no serious complications occured during the study. These results indicate that this technique has high efficacy, minimal complications, no systemic reaction, and a small number of imcomplete expulsions. Although this technique has a theoretical risk of infection from intrauterine retention of the balloon, this problem is only minimal. This may be due to the fact that patients with potential risk of infections were excluded from this study.

The mechanism of induction of abortion by condom-balloon is not clear, but the authors believe that mechanical separtation of membranes and decidua by condom-balloon initiates local prostaglandins because mechanical stimulation of this area results in endogenous prostaglandins production[7]. Separation of membranes and decidua may directly induce biochemical change in both tissues which contain a large amount of arachidonic acid and precursor of prostaglandins[1]. Uterine contractions induced by condom-balloon are similar to those of natural labour[1]. No significant systemic effect and no problem of overstimulation or tetanic contraction were observed.

The balloon did not separate placenta at all, always pushed in the direction of the decidua vera rather than the placental area when the actual procedure was visualized by sonography. Form our extensive experience, no placental abruption occured in any of the patients. However, patients with bleeding per vaginam should be sonographically exammined to rule out possible co-existing placenta previa which contraindicates the use of this technique.

Two interesting observations in condom-balloon technique are 1) most conceptive products are completely expulsed, only a few patients required further curettage due to incomplete expulsion, additionally, many patients delivered with intact membranes and 2) only a small amount of blood loss, possibly due to vasoconstriction from local prostaglandins.

In conclusion, this study demonstrates that the condom-balloon technique is highly effective for termination of DFU in the second trimester with minimal complications. In addition, this technique is much less expensive and should be appropriate for developing countries.

Acknowledgement

The authors wish to thank Associate Professor Kamjad Swadio, the Chairman of our Department, for his encouragement.

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Estimation of Fetal Weight in Utero by Ultrasound

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Abstract: *In order to find the best fitting regression equation to estimate fetal weight in utero using ultrasonic fetal parameters i.e. biparietal diameter (BPD), abdominal circumference(AC), and femur length(FL), ultrasonic measurements were carried out among 162 fetuses at Maharaj Nakorn Chiang Mai Hospital between February 1990 and June 1991. The ultrasonic measurements were conducted within 24 hours before delivery. The fetal weight was obtained from neonatal records. The data was analysed by using stepwise multiple linear regression analysis. It was found that the best fitting equation was: Log 10 (EFW) = 1.85479 + 0.09008(BPD) + 0.02466(AC) [coefficient of determination(R^2) = 0.939, multiple R = 0.969, p<0.000]. The absolute mean error ± SD(%) calculated by [estimated fetal weight - actual fetal weight / actual weight x 100] for the equation derived in this study group was 6.980 ± 7.017. The absolute mean error ± SD (%) for Shepard's equation applied to the fetal parameters from this study was 6.885 ± 6.397. Furthermore, a table of predicted fetal weight at various lengths of BPD and AC was prepared with computer assistance to read the estimated fetal weight directly (Thai J Obstet Gynaecol 1992;4: 15-22.)*

Key words: abdominal circumference(AC), biparietal diameter(BPD), estimated fetal weight(EFW), femur lenght(FL), ultrasound

An accurate assessment of fetal weight may be important in the management of a patient with preterm labour, intrauterine growth retardation (IUGR), premature rupture of membranes (PROM), macrosomia, or breech presentation. Perinatal mortality rates correlate with the accuracy of obstetric estimates of fetal weight. The importance of an accurate estimation has increased as improved neonatal care has lowered the gestational age of viability and increased the survival rates of low birth weight infants. Increasing attention is being paid to the accuracy of using various ultrasound measurements in estimating weight. Campbell et al[1] suggested that a single measurement of biparietal diameter (BPD) in late pregnancy was not clinically useful in assessing birth weight, and he reported that the use of

the abdominal circumference (AC) at the level of the ductus venosus was an accurate means of estimating fetal weight in utero[2]. Warsof et al[3], however, found that measurement of more than one parameter improved the correlation with birth weight. Since then, multiple fetal parameters for the prediction of fetal weight have been employed; these are the biparietal diameter, abdominal circumference, femur length (FL), total intrauterine volume (TIUV), etc..

The purpose of the present study was to 1) determine the best fitting regression equation to estimate fetal weight in utero using ultrasonic fetal parameter i.e. biparietal diameter (BPD) abdominal circumference (AC), and femur length (FL), and 2) compare the absolute mean error ± SD between our equations with Shepard's equation[4] which was commonly used in our institutes.

Materials and Methods

Sonographic measurements of BPD, AC, and FL were made in 162 live-born fetuses of Thai pregnant women within 24 hours before delivery, using commercially available linear-array real-time systems with 3.5 MHz transducers (Aloka SSD 650). Techniques of imaging and measurement have been described elsewhere[5-7]. BPD was measured from the outer margin of proximal skull table to the inner margin of the distal one[5] and FL from the greater trochanter to the distal metaphysis[6], using internal electronic calipers and speed of sound of 1540 m/sec in tissue. AC was measured along the outer boundaries of the abdomen at the level of the porto-umbilical vein complex[7]. The measurements were performed by two experienced perinatal sonographers who had no information about the patients and neonatal outcomes.

The case of study must meet the criteria of inclusion i.e. delivered within 24 hours after examination, live fetus, good quality of sonographic imaging, no brachycephaly or dolichocephaly of fetal head, no fetal anomalies.

The relationship between sonographic measurements and birth weight was evaluated by stepwise multiple regression analysis, which requires that all coefficents be significantly different from zero ($p \leq 0.05$). In order to be convenient for clinical application, the variables entered into the equation would be analyzed from one parameter and presented only the equation that R^2 or coefficient of determination were more than 0.90. We examined in order 1) one parameter :- AC, AC^2, BPD, BPD^2, FL, FL^2, 2) two parameters : AC, BPD (AC, BPD, AC^2 BPD^2, ACxBPD) AC, FL (AC, FL, AC^2, ACxFL) BPD, FL (BPD, FL, BPD^2, FL^2 BPDxFL), and 3) three parameters :-AC, BPD, FL, AC^2, BP D^2, FL^2, ACxFL, BPDxFL.

Calculated fetal weight by regression equation was presented in the formula : Log10(EFW)=a+b1X1+b2X2 +.....b9X9 (EFW=estimated fetal

weight, a=inter-cept, b1...b9= coefficient of X1.....X9 respectively, X1 .....X9=AC, BPD, AC^2, BPD^2, ACxBPD, FL, FL^2, ACxFL, BPDxFL, respectively).

The errors in prediction by the various equations were evaluated by absolute mean error (%) (=estimated fetal weight - actual weight/actual weight x 100).

Results

The sonographic parameters of 162 live-born fetuses which met the completed criteria were measured and born at Maharaj Nakorn Chiang Mai Hospital from February 1990 to June 1991.

Maternal age range was 16-44 years, average 27.6±4.95 years. 55.6% of them were primiparae.

Ultrasound fetal parameters from 162 fetuses are shown in Table 1.

Birth weight range was 500-4500 g, mean±SD=2236.97±738.71 g. 67.3% of the newborns were less than 2500 g and consisted of 83 male and 79 female babies.

By multiple linear regression analysis, five equations that R^2 were more than 0.90 and p value < 0.05 were formulated as shown below.

1. $\text{Log10(EFW)} = 1.28537 + 0.10436(AC) - 0.00116(AC)^2$
2. $\text{Log10(EFW)} = 1.85479 + 0.09008(BPD) + 0.02466(AC)$
3. $\text{Log10(EFW)} = 2.40549 + 0.002643(BPD)(AC) + 0.0325(BPD)$
4. $\text{Log10(EFW)} = 2.03714 + 0.0254(AC) + 0.08567(FL)$
5. $\text{Log10(EFW)} = 2.24784 + 0.09122(FL) + 0.002798(BPD)(AC) - 0.0010112(AC)(FL)$

R^2 and p value for each equation are shown in Table 2.

When we estimated the fetal weight from the sonographic parameters of the samples in this study by each equation and also Shepard's equation[4] [Log(EFW) = - 1.7492 + 0.166 (BPD) + 0.046(AC) - 2.646(ACxBPD) /1000] and then calculated absolute mean error(%) from actual weight. Of 5 equations in this study, equation was the best one and the absolute mean error was nearly the same as that of Shepard's equation[4].

Additionally, estimated fetal weight at various lengths of BPD and AC was calculated by equation 2 [Log 10(EFW) = 1.85479 + 0.09008(BPD)

Table 1 Sonographic parameters of the samples

Parameters	Numbers	Mean+SD (cm)	Range
BPD	162	8.30±0.77	5.0 - 9.7
AC	162	29.14±4.13	17.6 - 41.2
FL*	161	6.35±0.73	3.9 - 7.6

*FL could not be determined in one case.

Table 2 R^2 and p-value for each equation of estimated fetal weight

Equation	R^2	p-value
1	0.908	<0.000
2	0.939	<0.000
3	0.919	<0.000
4	0.919	<0.000
5	0.939	<0.000

Table 3 Absolute mean error for each equation

Equation	Absolute mean error+SD(%)
1	8.841 ± 7.816
2	6.980 ± 7.017
3	8.199 ± 8.081
4	8.344 ± 7.744
5	7.327 ± 6.568
Shepard's	6.885 ± 6.397

+ 0.02466(AC)] with computer assistance and presented in tabular form (Table 4).

Discussion

The knowledge of fetal weight in utero is important for obstetric decision making, especially in cases of preterm labour, abnormal fetal growth, breech presentation, macrosomia, etc., and is helpful in the follow-up of intrauterine fetal growth. This study was undertaken to determine the equation for estimation of fetal weight in utero. The equation was derived from various sonographic parameters obtained within 24 hours before delivery, i.e. BPD, AC, FL, and actual birth weight.

This study showed that the absolute mean errors of all equations were slightly different but equation 2 was the best, absolute mean error(%) = 6.980±7.017. The second best was equation 5. In spite of three incorporated parameters, the absolute mean error of equation 5 was slightly more than that of equation 2. This suggests that only BPD and AC were sufficient to predict the fetal size in utero, the incorporation of FL into various equations as a third parameter has not enhanced the accuracy of prediction. The extra time involved in the measurement of the third parameter is therefore unrewarding. Obviously, a quick, easy, repeatable measurement would be of advantage to a busy clinical practice. From this study, therefore, equation 2 was the most appropriate for clinical purpose use.

When we estimated the fetal weight from ultrasound parameters in this study using Shepard's equation[4], it was found that mean absolute error was very similar to that of equation 2. This finding indicates that racial factor may not influence equations of estimated fetal weight. When compared to previous Western studies[3, 4, 8-11] the absolute means error were not so different.

The factors providing reliability of this study include 1) the ultrasound parameters were measured by only two experienced perinatal sonographers, 2) the sonographers had no information of the patients and neonatal outcomes, 3) all measurements were

TABLE 4 Estimated fetal weight (grams) from BPD and AC from equation 2

BPD \ AC	15.5	16.0	16.5	17.0	17.5	18.0	18.5	19.0	19.5	20.0	20.5	21.0	21.5	22.0	22.5	23.0	23.5
3.1	328	338	347	357	368	378	389	400	412	424	436	449	462	475	489	503	517
3.2	335	345	355	365	375	386	397	409	421	433	445	458	471	485	499	513	528
3.3	342	352	362	373	383	394	406	417	429	442	455	468	481	495	509	524	539
3.4	349	359	370	380	391	403	414	426	438	451	464	477	491	505	520	535	550
3.5	357	367	378	388	400	411	423	435	448	461	474	487	501	516	531	546	562
3.6	364	375	385	397	408	420	432	444	457	470	484	498	512	527	542	558	574
3.7	372	383	394	405	417	429	441	454	467	480	494	508	523	538	553	569	586
3.8	380	391	402	413	425	437	450	463	476	490	504	519	534	549	565	581	598
3.9	388	399	410	422	434	447	460	473	486	500	515	530	545	561	577	593	610
4.0	396	407	419	431	443	456	469	483	497	511	526	541	556	572	589	606	623
4.1	404	416	428	440	453	466	479	493	507	522	537	552	568	584	601	618	636
4.2	412	424	437	449	462	475	489	503	518	533	548	564	580	597	614	631	650
4.3	421	433	446	459	472	485	499	514	528	544	559	575	592	609	627	645	663
4.4	430	442	455	468	482	495	510	524	540	555	571	587	604	622	640	658	677
4.5	439	452	465	478	492	506	520	535	551	567	583	600	617	635	653	672	691
4.6	448	461	474	488	502	516	531	547	562	579	595	612	630	648	667	686	706
4.7	458	471	484	498	513	527	542	558	574	591	608	625	643	662	681	700	721
4.8	467	481	494	509	523	538	554	570	586	603	620	638	657	676	695	715	736
4.9	477	491	505	519	534	550	565	582	598	616	633	652	670	690	710	730	751
5.0	487	501	515	530	545	561	577	594	611	629	647	665	685	704	725	745	767
5.1	497	511	526	541	557	573	589	606	624	642	660	679	699	719	740	761	783
5.2	507	522	537	553	569	585	602	619	637	655	674	694	714	734	755.	777	799
5.3	518	533	548	564	580	597	614	632	650	669	688	708	728	749	771	793	816
5.4	529	544	560	576	593	610	627	645	664	683	703	723	744	765	787	810	833
5.5	540	556	572	588	605	622	640	659	678	697	717	738	759	781	804	827	851
5.6	551	567	584	600	618	636	654	673	692	712	732	754	775	798	821	844	868
5.7	563	579	596	613	631	649	668	687	707	727	748	769	791	814	838	862	887
5.8	575	591	608	626	644	662	681	701	721	742	763	785	808	831	855	880	905
5.9	587	604	621	639	657	676	696	716	736	758	779	802	825	849	873	898	924
6.0	599	616	634	652	671	690	710	731	752	774	796	819	842	867	891	917	944
6.1	612	629	647	666	685	705	725	746	768	790	812	836	860	885	910	936	963
6.2	624	642	661	680	700	720	740	762	784	806	829	853	878	903	929	956	984
6.3	638	656	675	694	714	735	756	778	800	823	847	871	896	922	949	976	1004
6.4	651	670	689	709	729	750	772	794	817	840	865	890	915	942	969	997	1025
6.5	665	684	703	724	744	766	788	811	834	858	883	908	934	961	989	1017	1047
6.6	678	698	718	739	760	782	805	828	852	876	901	927	954	981	1010	1039	1069
6.7	693	713	733	754	776	798	821	845	869	894	920	947	974	1002	1031	1060	1091
6.8	707	728	749	770	792	815	839	863	888	913	939	966	994	1023	1052	1083	1114
6.9	722	743	764	786	809	832	856	881	906	932	959	987	1015	1044	1074	1105	1137
7.0	737	758	780	803	826	850	874	899	925	952	979	1007	1036	1066	1097	1129	1161
7.1	753	774	797	820	843	867	892	918	945	972	1000	1029	1058	1089	1120	1152	1185
7.2	768	791	813	837	861	886	911	937	964	992	1021	1050	1080	1111	1143	1176	1210
7.3	785	807	830	854	879	904	930	957	985	1013	1042	1072	1103	1135	1167	1201	1236
7.4	801	824	848	872	897	923	950	977	1005	1034	1064	1095	1126	1159	1192	1226	1262
7.5	818	841	866	890	916	942	970	998	1026	1056	1086	1118	1150	1183	1217	1252	1288
7.6	835	859	884	909	935	962	990	1018	1048	1078	1109	1141	1174	1208	1242	1278	1315
7.7	852	877	902	928	955	982	1011	1040	1070	1101	1132	1165	1198	1233	1268	1305	1343
7.8	870	895	921	948	975	1003	1032	1062	1092	1124	1156	1189	1224	1259	1295	1332	1371
7.9	888	914	940	967	995	1024	1053	1084	1115	1147	1180	1214	1249	1285	1322	1360	1399
8.0	907	933	960	988	1016	1045	1076	1107	1138	1171	1205	1240	1275	1312	1350	1389	1429
8.1	926	953	980	1008	1037	1067	1098	1130	1162	1196	1230	1266	1302	1340	1378	1418	1459
8.2	946	973	1001	1030	1059	1090	1121	1153	1187	1221	1256	1292	1329	1368	1407	1448	1489
8.3	965	993	1022	1051	1081	1113	1145	1178	1212	1246	1282	1319	1357	1396	1436	1478	1520
8.4	986	1014	1043	1073	1104	1136	1169	1202	1237	1273	1309	1347	1386	1426	1467	1509	1552
8.5	1006	1035	1065	1096	1127	1160	1193	1227	1263	1299	1337	1375	1415	1455	1497	1540	1585
8.6	1027	1057	1087	1119	1151	1184	1218	1253	1289	1326	1365	1404	1444	1486	1529	1573	1618
8.7	1049	1079	1110	1142	1175	1209	1244	1279	1316	1354	1393	1433	1475	1517	1561	1606	1652
8.8	1071	1102	1133	1166	1200	1234	1270	1306	1344	1383	1422	1463	1506	1549	1593	1639	1687
8.9	1093	1125	1157	1190	1225	1260	1296	1334	1372	1412	1452	1494	1537	1581	1627	1674	1722
9.0	1116	1148	1181	1215	1250	1286	1323	1362	1401	1441	1483	1525	1569	1614	1661	1709	1758
9.1	1140	1172	1206	1241	1277	1313	1351	1390	1430	1471	1514	1557	1602	1648	1696	1745	1795
9.2	1163	1197	1231	1267	1303	1341	1380	1419	1460	1502	1545	1590	1636	1683	1731	1781	1832
9.3	1188	1222	1257	1293	1331	1369	1408	1449	1491	1534	1578	1623	1670	1718	1768	1819	1871
9.4	1213	1248	1284	1321	1359	1398	1438	1479	1522	1566	1611	1657	1705	1754	1805	1857	1910
9.5	1238	1274	1311	1348	1387	1427	1468	1510	1554	1599	1645	1692	1741	1791	1842	1896	1950
9.6	1264	1301	1338	1377	1416	1457	1499	1542	1586	1632	1679	1727	1777	1828	1881	1935	1991
9.7	1291	1328	1366	1405	1446	1487	1530	1574	1620	1666	1714	1764	1814	1867	1921	1976	2033
9.8	1318	1356	1395	1435	1476	1519	1562	1607	1654	1701	1750	1801	1853	1906	1961	2017	2075
9.9	1345	1384	1424	1465	1507	1550	1595	1641	1688	1737	1787	1838	1891	1946	2002	2060	2119
10.0	1373	1413	1454	1496	1539	1583	1629	1675	1724	1773	1824	1877	1931	1987	2044	2103	2163

TABLE 4 Estimated fetal weight (grams) from BPD and AC (continued)

BPD \ AC	24.0	24.5	25.0	25.5	26.0	26.5	27.0	27.5	28.0	28.5	29.0	29.5	30.0	30.5	31.0	31.5	32.0
3.1	532	547	563	579	596	613	631	649	668	687	707	727	748	769	792	814	838
3.2	543	559	575	591	608	626	644	663	682	701	721	742	764	786	808	831	855
3.3	554	570	587	604	621	639	657	676	696	716	737	758	780	802	825	849	873
3.4	566	582	599	616	634	652	671	691	710	731	752	774	796	819	842	867	892
3.5	578	595	612	629	647	666	685	705	725	746	768	790	813	836	860	885	910
3.6	590	607	625	643	661	680	700	720	741	762	784	806	830	854	878	903	929
3.7	602	620	638	656	675	694	714	735	756	778	800	823	847	871	896	922	949
3.8	615	633	651	670	689	709	729	750	772	794	817	841	865	890	915	942	969
3.9	628	646	665	684	703	724	745	766	788	811	834	858	883	908	934	961	989
4.0	641	660	679	698	718	739	760	782	805	828	852	876	901	927	954	982	1010
4.1	655	673	693	713	733	754	776	798	821	845	869	895	920	947	974	1002	1031
4.2	668	688	707	728	749	770	792	815	839	863	888	913	940	967	994	1023	1053
4.3	682	702	722	743	764	786	809	832	856	881	906	932	959	987	1015	1045	1075
4.4	697	717	737	759	780	803	826	850	874	899	925	952	979	1008	1037	1066	1097
4.5	711	732	753	774	797	820	843	868	893	918	945	972	1000	1029	1058	1089	1120
4.6	726	747	769	791	813	837	861	886	911	937	964	992	1021	1050	1080	1112	1144
4.7	741	763	785	807	830	854	879	904	930	957	985	1013	1042	1072	1103	1135	1168
4.8	757	779	801	824	848	872	897	923	950	977	1005	1034	1064	1095	1126	1159	1192
4.9	773	795	818	841	866	891	916	943	970	998	1026	1056	1086	1118	1150	1183	1217
5.0	789	812	835	859	884	909	935	962	990	1019	1048	1078	1109	1141	1174	1208	1243
5.1	805	829	853	877	902	928	955	983	1011	1040	1070	1101	1132	1165	1199	1233	1269
5.2	822	846	870	895	921	948	975	1003	1032	1062	1092	1124	1156	1189	1224	1259	1295
5.3	840	864	889	914	941	968	995	1024	1054	1084	1115	1147	1180	1214	1249	1285	1322
5.4	857	882	907	933	960	988	1016	1046	1076	1107	1139	1171	1205	1240	1276	1312	1350
5.5	875	900	926	953	980	1009	1038	1068	1098	1130	1162	1196	1230	1266	1302	1340	1378
5.6	893	919	946	973	1001	1030	1059	1090	1121	1154	1187	1221	1256	1292	1330	1368	1407
5.7	912	938	965	993	1022	1051	1082	1113	1145	1178	1212	1247	1282	1319	1357	1396	1437
5.8	931	958	986	1014	1043	1073	1104	1136	1169	1202	1237	1273	1309	1347	1386	1426	1467
5.9	951	978	1006	1035	1065	1096	1127	1160	1193	1228	1263	1299	1337	1375	1415	1456	1498
6.0	971	999	1027	1057	1088	1119	1151	1184	1218	1253	1289	1327	1365	1404	1445	1486	1529
6.1	991	1020	1049	1079	1110	1142	1175	1209	1244	1280	1316	1354	1393	1434	1475	1517	1561
6.2	1012	1041	1071	1102	1134	1166	1200	1234	1270	1306	1344	1383	1423	1464	1506	1549	1594
6.3	1033	1063	1093	1125	1157	1191	1225	1260	1297	1334	1372	1412	1452	1494	1537	1582	1627
6.4	1055	1085	1116	1149	1182	1216	1251	1287	1324	1362	1401	1441	1483	1526	1570	1615	1661
6.5	1077	1108	1140	1173	1206	1241	1277	1314	1351	1390	1430	1472	1514	1558	1602	1649	1696
6.6	1099	1131	1164	1197	1232	1267	1304	1341	1380	1419	1460	1502	1546	1590	1636	1683	1732
6.7	1122	1155	1188	1222	1257	1294	1331	1369	1409	1449	1491	1534	1578	1624	1670	1718	1768
6.8	1146	1179	1213	1248	1284	1321	1359	1398	1438	1480	1522	1566	1611	1658	1705	1754	1805
6.9	1170	1204	1238	1274	1311	1348	1387	1427	1468	1511	1554	1599	1645	1692	1741	1791	1843
7.0	1195	1229	1264	1301	1338	1377	1416	1457	1499	1542	1587	1632	1679	1728	1778	1829	1881
7.1	1220	1255	1291	1328	1366	1406	1446	1488	1531	1575	1620	1667	1715	1764	1815	1867	1921
7.2	1245	1281	1318	1356	1395	1435	1476	1519	1563	1608	1654	1702	1751	1801	1853	1906	1961
7.3	1271	1308	1345	1384	1424	1465	1507	1551	1595	1641	1689	1737	1787	1839	1892	1946	2002
7.4	1298	1335	1374	1413	1454	1496	1539	1583	1629	1676	1724	1774	1825	1877	1931	1987	2044
7.5	1325	1363	1402	1443	1484	1527	1571	1616	1663	1711	1760	1811	1863	1917	1972	2029	2087
7.6	1353	1392	1432	1473	1516	1559	1604	1650	1698	1747	1797	1849	1902	1957	2013	2071	2131
7.7	1381	1421	1462	1504	1547	1592	1638	1685	1733	1783	1835	1887	1942	1998	2055	2114	2175
7.8	1410	1451	1492	1535	1580	1625	1672	1720	1770	1821	1873	1927	1983	2040	2098	2159	2221
7.9	1440	1481	1524	1568	1613	1659	1707	1756	1807	1859	1912	1967	2024	2082	2142	2204	2267
8.0	1470	1512	1556	1601	1647	1694	1743	1793	1845	1898	1952	2009	2066	2126	2187	2250	2315
8.1	1501	1544	1588	1634	1681	1730	1779	1831	1883	1938	1993	2051	2110	2171	2233	2297	2364
8.2	1532	1576	1622	1668	1716	1766	1817	1869	1923	1978	2035	2094	2154	2216	2280	2346	2413
8.3	1564	1609	1656	1703	1752	1803	1855	1908	1963	2020	2078	2138	2199	2262	2328	2395	2464
8.4	1597	1643	1690	1739	1789	1841	1894	1948	2004	2062	2121	2182	2245	2310	2376	2445	2515
8.5	1630	1677	1726	1775	1827	1879	1933	1989	2046	2105	2166	2228	2292	2358	2426	2496	2568
8.6	1665	1713	1762	1813	1865	1919	1974	2031	2089	2149	2211	2275	2340	2408	2477	2548	2622
8.7	1700	1748	1799	1851	1904	1959	2015	2073	2133	2194	2257	2323	2389	2458	2529	2602	2677
8.8	1735	1785	1837	1889	1944	2000	2057	2117	2178	2240	2305	2371	2439	2510	2582	2656	2733
8.9	1772	1823	1875	1929	1985	2042	2100	2161	2223	2287	2353	2421	2491	2562	2636	2712	2790
9.0	1809	1861	1914	1969	2026	2084	2145	2206	2270	2335	2402	2472	2543	2616	2691	2769	2849
9.1	1847	1900	1954	2011	2069	2128	2189	2253	2317	2384	2453	2523	2596	2671	2748	2827	2908
9.2	1885	1940	1995	2053	2112	2173	2235	2300	2366	2434	2504	2576	2650	2727	2805	2886	2969
9.3	1925	1980	2037	2096	2156	2218	2282	2348	2416	2485	2557	2630	2706	2784	2864	2947	3031
9.4	1965	2022	2080	2140	2201	2265	2330	2397	2466	2537	2610	2685	2763	2842	2924	3008	3095
9.5	2006	2064	2123	2185	2248	2312	2379	2447	2518	2590	2665	2742	2821	2902	2985	3071	3160
9.6	2048	2107	2168	2230	2295	2361	2429	2499	2571	2645	2721	2799	2880	2963	3048	3136	3226
9.7	2091	2151	2213	2277	2343	2410	2480	2551	2624	2700	2778	2858	2940	3025	3112	3202	3294
9.8	2135	2197	2260	2325	2392	2461	2532	2604	2680	2757	2836	2918	3002	3088	3177	3269	3363
9.9	2180	2243	2307	2374	2442	2512	2585	2659	2736	2814	2895	2979	3065	3153	3244	3337	3433
10.0	2226	2290	2356	2423	2493	2565	2639	2715	2793	2873	2956	3041	3129	3219	3312	3407	3505

TABLE 4 Estimated fetal weight (grams) from BPD and AC (continued)

BPD \ AC	32.5	33.0	33.5	34.0	34.5	35.0	35.5	36.0	36.5	37.0	37.5	38.0	38.5	39.0	39.5	40.0
3.1	862	887	912	939	966	993	1022	1051	1082	1113	1145	1178	1212	1247	1283	1320
3.2	880	905	931	958	986	1014	1043	1074	1104	1136	1169	1203	1237	1273	1310	1347
3.3	898	924	951	978	1007	1036	1065	1096	1128	1160	1193	1228	1263	1300	1337	1375
3.4	917	944	971	999	1028	1057	1088	1119	1151	1184	1218	1254	1290	1327	1365	1404
3.5	937	963	991	1020	1049	1079	1110	1142	1175	1209	1244	1280	1317	1355	1394	1434
3.6	956	984	1012	1041	1071	1102	1134	1166	1200	1235	1270	1307	1344	1383	1423	1464
3.7	976	1004	1033	1063	1094	1125	1157	1191	1225	1260	1297	1334	1372	1412	1453	1494
3.8	997	1025	1055	1085	1117	1149	1182	1216	1251	1287	1324	1362	1401	1442	1483	1526
3.9	1018	1047	1077	1108	1140	1173	1207	1241	1277	1314	1352	1391	1431	1472	1514	1558
4.0	1039	1069	1100	1131	1164	1197	1232	1267	1304	1341	1380	1420	1461	1503	1546	1590
4.1	1061	1091	1123	1155	1188	1222	1258	1294	1331	1369	1409	1449	1491	1534	1578	1624
4.2	1083	1114	1146	1179	1213	1248	1284	1321	1359	1398	1438	1480	1522	1566	1611	1658
4.3	1106	1137	1170	1204	1239	1274	1311	1349	1387	1427	1469	1511	1554	1599	1645	1693
4.4	1129	1161	1195	1229	1264	1301	1338	1377	1417	1457	1499	1542	1587	1633	1680	1728
4.5	1152	1186	1220	1255	1291	1328	1366	1406	1446	1488	1531	1575	1620	1667	1715	1764
4.6	1177	1210	1245	1281	1318	1356	1395	1435	1477	1519	1563	1608	1654	1702	1751	1801
4.7	1201	1236	1271	1308	1346	1384	1424	1465	1507	1551	1596	1642	1689	1737	1787	1839
4.8	1226	1262	1298	1335	1374	1413	1454	1496	1539	1583	1629	1676	1724	1774	1825	1877
4.9	1252	1288	1325	1363	1403	1443	1485	1527	1571	1617	1663	1711	1760	1811	1863	1917
5.0	1278	1315	1353	1392	1432	1473	1516	1559	1604	1650	1698	1747	1797	1849	1902	1957
5.1	1305	1343	1381	1421	1462	1504	1547	1592	1638	1685	1734	1784	1835	1888	1942	1998
5.2	1332	1371	1410	1451	1493	1536	1580	1625	1672	1720	1770	1821	1873	1927	1983	2040
5.3	1360	1400	1440	1481	1524	1568	1613	1659	1707	1756	1807	1859	1913	1968	2024	2083
5.4	1389	1429	1470	1512	1556	1601	1647	1694	1743	1793	1845	1898	1953	2009	2067	2126
5.5	1418	1459	1501	1544	1589	1634	1681	1730	1780	1831	1884	1938	1994	2051	2110	2171
5.6	1448	1489	1532	1576	1622	1669	1717	1766	1817	1869	1923	1978	2035	2094	2154	2216
5.7	1478	1521	1564	1609	1656	1704	1753	1803	1855	1908	1963	2020	2078	2138	2199	2263
5.8	1509	1553	1597	1643	1691	1739	1789	1841	1894	1948	2004	2062	2122	2183	2246	2310
5.9	1541	1585	1631	1678	1726	1776	1827	1879	1934	1989	2047	2105	2166	2228	2293	2359
6.0	1573	1618	1665	1713	1762	1813	1865	1919	1974	2031	2089	2150	2211	2275	2341	2408
6.1	1606	1652	1700	1749	1799	1851	1904	1959	2015	2073	2133	2195	2258	2323	2390	2459
6.2	1640	1687	1735	1785	1837	1890	1944	2000	2058	2117	2178	2241	2305	2372	2440	2510
6.3	1674	1722	1772	1823	1875	1929	1985	2042	2101	2161	2224	2288	2353	2421	2491	2563
6.4	1709	1758	1809	1861	1915	1970	2026	2085	2145	2207	2270	2336	2403	2472	2543	2616
6.5	1745	1795	1847	1900	1955	2011	2069	2128	2190	2253	2318	2384	2453	2524	2596	2671
6.6	1781	1833	1886	1940	1996	2053	2112	2173	2236	2300	2366	2434	2505	2577	2651	2727
6.7	1819	1871	1925	1980	2037	2096	2157	2219	2283	2348	2416	2485	2557	2631	2706	2784
6.8	1857	1910	1965	2022	2080	2140	2202	2265	2330	2397	2467	2538	2611	2686	2763	2843
6.9	1896	1950	2007	2064	2124	2185	2248	2313	2379	2448	2518	2591	2665	2742	2821	2902
7.0	1936	1991	2049	2108	2168	2231	2295	2361	2429	2499	2571	2645	2721	2800	2880	2963
7.1	1976	2033	2092	2152	2214	2277	2343	2411	2480	2551	2625	2700	2778	2858	2941	3025
7.2	2018	2076	2135	2197	2260	2325	2392	2461	2532	2605	2680	2757	2836	2918	3002	3089
7.3	2060	2119	2180	2243	2308	2374	2442	2513	2585	2659	2736	2815	2896	2979	3065	3153
7.4	2103	2164	2226	2290	2356	2424	2494	2565	2639	2715	2793	2874	2957	3042	3129	3219
7.5	2147	2209	2272	2338	2405	2475	2546	2619	2695	2772	2852	2934	3019	3105	3195	3287
7.6	2192	2255	2320	2387	2456	2526	2599	2674	2751	2830	2912	2996	3082	3171	3262	3356
7.7	2238	2302	2369	2437	2507	2579	2654	2730	2809	2890	2973	3058	3146	3237	3330	3426
7.8	2285	2351	2418	2488	2560	2633	2709	2787	2868	2950	3035	3122	3212	3305	3400	3498
7.9	2333	2400	2469	2540	2613	2689	2766	2846	2928	3012	3099	3188	3280	3374	3471	3571
8.0	2382	2450	2521	2593	2668	2745	2824	2905	2989	3075	3164	3255	3348	3445	3544	3646
8.1	2432	2502	2574	2648	2724	2802	2883	2966	3052	3139	3230	3323	3419	3517	3618	3723
8.2	2483	2554	2628	2703	2781	2861	2944	3028	3116	3205	3298	3393	3490	3591	3694	3801
8.3	2535	2608	2683	2760	2839	2921	3005	3092	3181	3272	3367	3464	3563	3666	3772	3880
8.4	2588	2662	2739	2818	2899	2982	3068	3157	3248	3341	3437	3536	3638	3743	3851	3962
8.5	2642	2718	2796	2877	2960	3045	3133	3223	3316	3411	3509	3610	3714	3821	3931	4045
8.6	2697	2775	2855	2937	3022	3109	3198	3290	3385	3483	3583	3686	3792	3901	4014	4129
8.7	2754	2833	2915	2999	3085	3174	3265	3359	3456	3556	3658	3763	3872	3983	4098	4216
8.8	2812	2893	2976	3061	3150	3240	3334	3430	3528	3630	3735	3842	3953	4067	4184	4304
8.9	2870	2953	3038	3126	3216	3308	3404	3502	3602	3706	3813	3923	4036	4152	4271	4394
9.0	2931	3015	3102	3191	3283	3378	3475	3575	3678	3784	3893	4005	4120	4239	4361	4487
9.1	2992	3078	3167	3258	3352	3448	3548	3650	3755	3863	3974	4089	4207	4328	4452	4581
9.2	3055	3143	3233	3326	3422	3521	3622	3726	3834	3944	4058	4175	4295	4418	4546	4677
9.3	3119	3209	3301	3396	3494	3594	3698	3804	3914	4027	4143	4262	4385	4511	4641	4775
9.4	3184	3276	3370	3467	3567	3670	3775	3884	3996	4111	4230	4351	4477	4606	4738	4875
9.5	3251	3344	3441	3540	3642	3747	3855	3966	4080	4197	4318	4443	4570	4702	4837	4977
9.6	3319	3415	3513	3614	3718	3825	3935	4049	4165	4285	4409	4536	4666	4801	4939	5081
9.7	3389	3486	3587	3690	3796	3905	4018	4134	4253	4375	4501	4631	4764	4901	5042	5188
9.8	3460	3559	3662	3767	3876	3987	4102	4220	4342	4467	4595	4728	4864	5004	5148	5296
9.9	3532	3634	3738	3846	3957	4071	4188	4309	4433	4560	4692	4827	4966	5109	5256	5407
10.0	3606	3710	3817	3927	4040	4156	4276	4399	4526	4656	4790	4928	5070	5216	5366	5521

examined with the same scanner, 4) all newborns were weighed by the same equipment, and 5) the measurement was done within 24 hours of delivery, unlike many previous studies.

The limitations of this study may include 1) variations in measurements between the two sonographers, 2) the measurements may be erroneous in cases of obesity or engaged fetal head, and 3) no comparison between sonographic parameters and actual neonatal ones, thererfore, systematic error was not known.

However, the equation from this study should be further tested in another sample to evaluate the precision and accuracy of prediction.

Acknowledgement

The authors wish to thank Associate Professor Sungwal Rugpao for her valuable help with the statistical analysis. We also wish to thank Associate Professor Kamjad Swadio and Associate Professor Suri Simarak for their encouragement.

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Preliminary Experiences with IVF & ET at Maharaj Nakorn Chiang Mai Hospital

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Abstract : *This is the first report of 35 patients who registered for IVF-ET at Maharaj Nakorn Chiang Mai Hospital from January 1 to September 30, 1991. The patients' ages ranged from 29-42 years, with a duration of infertility of 2-16 years. Gonadotropin releasing hormone agonist and human menopausal gonadotropins were prescribed for superovulation. Follicular growth was monitored by serial measurement of serum estradiol levels and pelvic ultrasound. Human chorionic gonadotropin was given intramuscularly when sonogram demostrated two or more follicles ≥ 17 mm in diameter. GnRH-a was discontinued and oocyte retrieval was performed 34-36 hours later. Sperm was prepared by the washing and swim-up technique. Mature oocyte was inseminated with 100000 motile sperms and was observed for evidence of fertilization 12-16 hours later. Embryo transfer was performed 48-72 hours after oocyte was retrieval.*

Of the 336 harvested oocytes, 183 (55.3%) achieved fertilization. Embryo transfer was done in 37 cycles at 2- to 8-cell stages and resulted in 4 clinical pregnancies (10.8% of ET). Two pregnancies ended in spontaneous miscarriages at 11 weeks of gestation, the other two are well on-going. (Thai J Obstet Gynaecol 1992;4: 23-32.)

Key words : IVF & ET, preliminary experience

Steptoe and Edwards[1] reported the birth of the first baby after *in vitro* fertilization and embryo transfer (IVF-ET) in 1978. It opened a new era in the treatment of infertility. Today, many clinics around the world are practicing human IVF with varying degrees of success. In this report, we describe our preliminary experience with this technique at Maharaj Nakorn

Chiang Mai Hospital.

Materials and Methods

Setting of the unit

Maharaj Nakorn Chiang Mai Hospital is the only University Hospital in Northern Thailand and a major tertiary care center. The team of assisted reproduction consists of four full-time reproductive endocrinologists and infertility specialists with two full-time scientists. All hormonal assays are done in the human reproduction laboratory, using commercial radioimmunoassay kits. Oocyte/embryo culture and medium preparation are done in another separate laboratory, which is located in a semi-sterile area inside the delivery suite. The culture laboratory is within a short walking distance from the operating room where oocyte pick-up is routinely performed. Media preparations, dilutions and transfers are done under a vertical laminar flow hood. Ham's F-10 medium is used in our system. The medium is prepared once a week by dissolving 0.981 g of F-10 nutrient mixture powder (GIBCO, New York, Cat. No. 430-1200 EB) in 100 ml of ultra-pure (HPLC-grade) water (SeroMed®, Berlin) in a sterile disposable tissue culture flask (Nunclon®, Denmark). To this are added the followings: calcium lactate 0.0308 g, sodium bicarbonate 0.2106 g, penicillin-G sodium 0.0075 g and streptomycin sulfate 0.0075 g (Sigma cell culture reagents®, USA). The culture medium is checked for osmolarity, which invariably works out between 280 and 285 milliosmol/kg. The culture medium is then filtered through disposable syringe filter (Minisart® 0.2 μm, Sartorius) using a sterile 20-ml disposable syringe. Disposable 4-well multidish (Nunclon®, Denmark) is used for culture of gametes and embryos. Diposable 25 and 50 μl micropipettes (Clay Adams, NJ) are packed individually in aluminium foil and sterilized by heat in a hot air oven. These micropipettes are used to transfer harvested oocytes/embryos instead of Pasteur pipettes. Other glassware and equipment which come into contact with gametes are rinsed with distilled-deionised water and soaked in 1% hydrochloric acid solution for 60 minutes, then flushed through with distilled-deionised water, soaked in hot 1% solution of "7X" tissue culture detergent for 10 minutes, rinsed three times in distilled-deionised water and soaked overnight with ultra-pure water before sterilization by dry heat at 120° C for 6 hours.

Patient selection

Pretreatment screening involved a review of relevant medical history, a complete physical examination of the couples and basic infertility investigations which included at least two semen analyses; confirmation of ovulation and corpus luteum function with endometrial biopsy or mid-luteal serum progesterone assay: post-coital tests and laparoscopic tubal patency tests. A hysterosalpingogram was not

routinely required prior to treatment. Patients with tubal problems were treated surgically. Oligospermic males, defined as a sperm count of less than 20 x 10^6/ml, were treated with mesterolone acetate for 6 months, with or without split-ejaculate and intrauterine insemination. If these conventional therapies were unsuccessful, they were considered for IVF-ET after a detailed discussion of the procedure.

Controlled ovarian hyperstimulation

In our clinic, both long and short ovarian stimulation protocols, as described by others[2] are in use. The allocation of patients to these two treatments was made at the discretion of the attending physicians, and not randomly. In brief, buserelin acetate (Suprefact®, Hoechst) was administered intranasally at a dose of 100 μg six times per day, starting in the mid-luteal phase of the preceding cycle in the long protocol, or on the first day of the current cycle in the short protocol. In both protocols, human menopausal gonadotropins (hMG 75 IU, Pergonal®, Serono) were given 2-4 ampoules intramuscularly, according to patients' age and previous responses, from cycle day 3 onward. Pelvic sonogram was done on the first day of the cycle as baseline, and then on a daily basis from day 8 of the cycle to monitor follicular growth. Daily measurement of serum estradiol by radioimmunoassay was done from day 6 of the cycle. Human chorionic gonadotropin (hCG, Profasi®, Serono) 10000 IU was given intramuscularly when ultrasound demonstrated two or more ovarian follicles exceeding 17 mm in diameter and estradiol level reached ≥ 300 pg/ml per dominant follicle. Buserelin was stopped on the day of hCG injection and oocyte retrieval was scheduled 34-36 hours later.

Oocyte recovery

Transvaginal oocyte retrieval under ultrasonic guidance was performed on an out-patient basis. The procedure was done in the morning after at least 6 hours of NPO, under intravenous pethidine (75mg) and diazepam (10mg) sedation. The analgesic and sedative were given just prior to the operation. The patient was placed in the lithotomy position and draped as in vaginal operation. The vagina was then thoroughly cleansed with normal saline solution. The 5-MHz vaginal ultrasonic transducer was covered with a sterile rubber glove. To improve the ultrasound image, contact jelly was placed inside between the transducer and the glove. A specially designed needle guide was then attached to the transducer and the transducer was connected to an Aloka SSD-650 ultrasound scanner with a viewing monitor. In the puncture mode, a dotted line representing the needle path was displayed on the monitor. A disposable double-lumen 16-gauge, 30 cm long needle with echogenic tip (Cook, Australia) was used for follicular aspiration. The

needle was rinsed with heparinized phosphate buffer saline (PBS) before being connected to the suction system.

The vaginal probe, with the needle guide in place, was introduced into the vagina. The pelvis was scanned and the number and size of follicles noted. Usually, we aspirated the most proximal follicles that were located in line with the needle path first. After determining and selecting the angle of puncture, the needle was placed in the needle guide and advanced into the follicle with a sharp jabbing action. Negative pressure of 100 mmHg was applied to collapse the follicle under vision on the ultrasound monitor. During aspiration, the needle was gently rotated and moved in all directions around the inner circumference of the follicle to give a curettage effect which increased the chance of oocyte collection. We routinely flushed each follicle twice with heparinized PBS to ensure recovery of oocyte. The flush volume used was approximately three-fourths of the original follicle volume. The operator then moved onto the next follicles and the same procedure was repeated until all follicles larger than 1 cm had been aspirated. The patient was observed for 2 hours and then discharged home on doxycycline 200mg/day for 5 days as prophylactic antibiotic.

Fluid from follicular puncture was examined carefully to recover oocytes under a dissecting microscope. Each harvested oocyte was placed in 1 ml of Ham's F-10 supplemented with 10% patients's serum in a 4-well plate, and kept in an incubator under 5% CO_2 in air at 37° C.

Sperm preparation

Male partners were requested to produce semen samples after oocyte retrieval. Semen was allowed to liquefy for 30 minutes at room temperature before analysis, and the results were recorded on a standard form. Semen was then gently mixed with Ham's F-10 at a ratio of 1:2 (vol/vol) and centrifuged for 10 minutes at 300xg. The supernatant was discarded and the sperm pellet resuspended in 1 ml of medium. After a second wash, 0.5 ml of medium was layered onto the loosened pellet and motile sperm were allowed to swim up for 45 minutes in a 5% CO_2 incubator at 37° C. Sperm concentration in the supernatant was reassessed and adjusted to 100000/25 µl.

Oocyte insemination

After 4 hours of preincubation, mature oocytes were inseminated with 100000 actively motile spermatozoa and left undisturbed at 37° C in a humidifed atmosphere of 5% CO_2 in air. After 12-16 hours, the ova were observed for evidence of fertilization and were transferred into fresh medium. Unfertilized oocytes were reinseminated in the same way.

Embryo transfer

The embryos at 2- to 8-cell

stages were transferred into the uterine cavity using Tom Cat catheter 48-72 hours after oocyte retrieval. In brief, the patients were placed in the lithotomy position. The cervix was exposed with a bivalve speculum and the ectocervix was gently cleansed with normal saline solution. The embryos were loaded into the catheter with 30 µl of culture medium supplemented with 50% patient's serum. The catheter was passed through the cervical canal into the uterine cavity until it reached the fundus. The catheter was then slightly withdrawn and the embryos were expelled. The catheter was gently withdrawn and promptly checked under a microscope to ensure that the embryos had not been retained. The patients were placed in Trendelenberg's position for 30 minutes and then discharged home.

Luteal phase support

All patients received hCG (Pregnyl®, Organon) 1500 IU intramuscularly on the day of embryo transfer and on days 3,6 and 9 after the transfer.

Diagnosis of pregnancy

Pregnancy was diagnosed on day 14 post-embryo transfer when the level of serum ß-hCG was greater than 25 mIU/ml, followed by a higher level in a subsequent assay 2 days later (biochemical pregnancy). Clinical pregnancy was diagnosed when a gestational sac was visualized under vaginal ultrasound, which should be clearly seen from day 35 post-embryo transfer.

Results

Thirty-eight couples were enrolled for 46 cycles of IVF-ET. There were eight patients who underwent the IVF procedure twice. Of the 46 cycles, three cycles in three patients were cancelled before oocyte retrieval, two because of poor ovarian response to hMG and one because of decreasing estradiol level before oocyte pick-up.

The average age of the remaining thirty five patients was 31 years (ranged 29-42 years). Twenty eight of them had primary and seven had secondary infertility, for an average duration of 6.8 years (ranged 2-16 years). Their infertility diagnoses are shown in Table 1. One couple had both tubal obstruction and male factor infertility. Long ovulation induction protocols were used in 26 cycles and short protocols in 17 cycles. On the average, 29.7 ampoules of hMG were needed in the long protocol versus only 21.2 ampoules in the short protocol. Serum estradiol (E2) levels before hCG administration and the number of oocytes harvested were significantly higher in the long than in the short protocols (Table 2). Prewashed sperm motility increased from 51.9% to 81% after washing.

Three hundred and thirty one oocytes were harvested, but only 183 achieved fertilization, giving an overall

fertilization rate of 55.3%. Low fertilization rates were observed in older couples and in couples with male factor infertility. Unfortunately, we did not keep detailed records of oocytes quality to allow meaningful correlation with fertilization rates. In our series slightly less than 10% of reinseminated oocytes were fertilized. Embryo transfer was done 48-72 hours after oocyte pick-up, when the embryos reached the 2- to 8-cell stages (Table 3).

The average number of embryos replaced was 4.9 (ranged 2-10) and resulted in 4 clinical pregnancies (10.8% of ET). All pregnancies occurred in patients with tubal obstruction and none in the group with male factor infertility. The characteristics of pregnant cycles are shown in Table 4.

Table 1 Infertility diagnosis

Diagnosis	No.
Tubal obstruction	32
Male factor	4

Table 3 Stages of embryo development at the time of embryo transfer

Stages of embryo	Number
Pronuclear	3
2-cell	20
3-cell	8
4-cell	132
5-cell	2
6-cell	5
8-cell	13
Total	183

Two patients with singleton pregnancies had spontaneous miscarriages at 11 weeks 4 days and 11 weeks 2 days gestation. Chromosome study of the first abortus was normal 46 XX and the other was trisomy-21. The remaining two pregnancies are well on-going. One is a singleton pregnancy and the other is a triplets, at 22 and 10 weeks gestation respectively.

Discussion

Although indications for IVF-

Table 2 Outcome of IVF-ET

	Long protocol	Short protocol	P*
Number of cycles	26	17	-
Number of hMG used (ampoules)	29.7 ± 12.8	21.2 ± 10.7	< 0.02
Serum E2 level (pg/ml)	2173.1 ± 880.3	1391.2 ± 543.5	< 0.002
Number of oocytes harvested	9.7 ± 6.1	5.5 ± 3.4	< 0.01
Pregnancy	3	1	-

* Student's t-test was used for analysis

Table 4 Characteristics of pregnant cycles

Women's Age	Men's Age	E2# at OPU	hMG* (amp)	Oocytes retrieved	Oocytes fertilized	Embryos transferred	Stages			
							2-cell	3-cell	4-cell	8-cell
35@	36	1550	24	8	6	6	1	2	3	-
33$	36	1300	24	7	5	5	1	-	4	-
30^	32	2500	16	14	12	10	1	-	5	4
35	37	2000	16	4	4	4	1	-	2	1

\# E2 in pg/ml, OPU = oocyte pick-up
* One ampoule of hMG contains 75 IU FSH
@ Triplet pregnancy
$ Abortion with Down's syndrome
^ Spontaneous abortion at 11 weeks gestation

ET in our series of patients are limited only to tubal obstruction and male factor infertility, others[3,4] have extended their indications to treat patients with other causes of infertility as a therapy of final resort in conditions such as hostile cervical mucus, immunologic infertility, ovulatory disorder, endometriosis, unexplained infertility and in ovum donation.

It has been customary for most IVF programs not to offer treatment of patients above the age of 40[5]. We began with the same rule in respect of age. However, this rule was occasionally violated to accommodate patients who persistently request IVF as a therapy of final resort and could afford the cost of treatment. In our series, there were two such patients who were willing to try IVF despite the known fact of a diminished success for this age group. One of them had poor response to hMG and the cycle was cancelled prior to oocyte retrieval, the other ended up in embryo transfer but did not become pregnant. In view of the fact that pregnancies from IVF have been reported in patients at age 40 and above[5], we believe it is justified to consider IVF in a few selected patients around the age of 40 years.

In most instances, there is no effective therapy for infertility[5]. With the discovery that relatively few spermatozoa were required for IVF, it was proposed that subnormal semen samples could be used to fertilize in vitro if sufficient numbers of spermatozoa could be obtained[6]. In our series, couples with male factor experienced very low fertilization rate and no pregnancy occurred in this group. However, others have reported success with IVF for the treatment of male infertility[7]. With the promise of newer techniques, such as zona drilling, sperm microinjection and sperm stimulation still on the horizon, IVF may be of major use in the future treatment of male infertility[8].

In our study, significantly more ampoules of hMG were needed in the long versus the short protocol, probably reflecting the absence of endogenous pituitary contribution when hMG stimulation was begun after down regulation has been accomplished[2]. Moreover, the number of oocytes harvested was also significantly higher in the long than in the short protocol. Our data seem to support Mordel et al[9], who reported that only the long protocol achieved significantly better result over hMG-only stimulated cycles in the number of aspirated oocytes. However, we would like to point out that the observed differences in our results could have been influenced by other confounding factors because patient allocation to either the short or long protocol was not made at random. In a study by Frydman et al[10], they could not demonstrate any advantage of long versus short protocol regarding folliculogenesis, oocytes recovered and pregnancy rates. Until the advantage of either protocol is established, we feel that the choice should be tailored according to the patients' convenience, cost and side effects.

Observations from many clinics have been that the incidence of pregnancy increases with the number of embryos replaced[11]. However, because of the concern about multiple pregnancies of higher orders (≥ triplets), many fertility centers restrict the number of embryos transferred to 3 or 4[11,12]. In so doing, difficulty arises in the selection of quality embryos. To date a precise method to determine the quality of human conceptus is not available. So far, cleavage rates and morphological criteria have been only rough indicators of viability.[13] Pregnancy has been established after transfer at 2-, 4-, 6-, 8-, 16-cell morula or blastocyst stage of concepti; homogeneous and regular blastomeres implant, as well as those with blebs and granular aspects[13]. To by-pass this technical problem and to avoid the ethical dilemma of discarding surplus human embryos, we decided to replace all fertilized ova unless the couples specify otherwise such as donation to other couples. We agree that in places where cryofacilities are available, it is advisable to replace fewer embryos for each cycle, with consideration given to cryopreservation of excess embryos for transfer in subsequent cycles.

Embryo transfer is an important step which requires skill and patience. Any delay or difficulty in entering or passing through the cervical canal can result in changes in the composition of the culture medium. This, in turn, may jeopardize the embryos and reduce the chance of implantation[14]. Steptoe et al[14] suggested that the use of a single cannula instead of the outer sleeve and inner cannula made the placement of embryo easier, which is in agreement with our experience using Tom Cat catheter. In our series, we encountered difficulty in passing the catheter into the uterine cavity in 5 patients and had to dilate the cervix using the

smallest Hegar's dilator.

In this report, the pregnancy rate is only 10.8% per embryo transfer, which is relatively low compared to other well-established centers[4]. This may be partly due to our lack of expertise in this relatively new field of assisted reproduction. However, we hope to achieve a better result in the future.

Acknowledgement

The authors wish to thank Associate Professor Dr. Kamjad Swasdio, Chairman of the Department of Obstetrics and Gynaecology and Professor Dr. Kosin Amatayakul for their continuing encouragement and support, Assistant Professor Dr. Wunnee Ojarasporn for her excellent technical assistance in ultrasonography and colleagues in the Department of Obstetrics and Gynaecology, and Radiology for their advices and co-operations.

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Addendum :

Since the first submission of this manuscript, we have achieved 4 more IVF pregnancies (as of February 7, 1992), giving the current pregnancy rates of 8/67 = 11.9% per cycle or 8/59 = 13.5% per embryo transfer. The first IVF baby in Northern Thailand was born by caesarean section at Maharaj Nakorn Chiang Mai Hospital on January 7, 1992. It was a boy, weighing 3680 g. The triplets in this report is now at 28 weeks of gestation.

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Vaginal Bleeding Patterns Among Lactating Women Using Contraceptive Methods

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Abstract : *Menstrual diary records were obtained from 322 lactating women, each for one year duration starting from delivery. The women were divided into 4 groups according to types of contraceptive used i.e. 114 Depo-provera, 99 Norplant, 32 IUDs and 77 tubal sterilization. A comparative analysis of their vaginal bleeding patterns was performed. The analytic procedures involved dividing each subject's diary into 4 successive 90-day reference periods, and indices for each period were calculated. Depo-provera and Norplant had a similar number of bleeding/spotting episodes. The median values of bleeding/spotting episodes for either group were 1,0,0 and 0 for reference period 1,2,3 and 4, respectively. Median values of bleeding/spotting episodes for each reference period for women using IUDs were 1,2,3 and 3; and for women undergoing tubal sterilization were 0, 0.1 and 2. Median values of number of bleeding/spotting days for reference period 1,2,3 and 4 were as follows : Depo-provera 9.0, 0.0, 0.0 and 0.0 ; Norplant 5.0, 0.0, 0.0 and 0.0; IUDs 4.0, 8.0, 9.0 and 10.5; and tubul sterilization 0.0, 0.0, 0.5 and 7.0. (Thai J Obstet Gynaecol 1992;4: 33-41.)*

Key words : vaginal bleeding patterns, lactating women, contraceptives

Although lactational amenorrhea has been regarded as a good method of fertility regulation particularly when other family planning methods are not readily available or desirable, it is common practice in Thailand to start temporary contraceptives at six weeks postpartum. Among hormonal contraceptives, progestogen only contraceptives, either in injectable or implant forms, which have not been shown to have any adverse effect on lactation and may even augment milk production, are popular[1,2]. Some ex-

perts recommend that progestogens, rather than combined oral contraceptives, be used by breast-feeding women[3,4]. Disturbance in vaginal bleeding induced by methods of contraception is an important side effect because of its potential impact on acceptability and continuation rate. Knowledge of these changes is essential for effective counselling.

Most studies concerning the influence of contraceptives on the vaginal bleeding pattern were concentrated on non-lactating women[5,6].

The objective of this paper is to study the influence of progestogen only contraceptives (Depo - provera and Norplant) on vaginal bleeding patterns among lactating women by comparing them with lactating women who had undergone abdominal tubal sterilization (TR) or who were fitted with Multiload Cu250 IUDs.

Materials and Methods

Women who fulfilled the following criteria were recruited to the study: 18-35 years old; having had vaginally delivered term single infants; having breast fed and intending to breast feed for at least 6 months; and intending to use one of the following contraceptive methods: Depo - provera (DMPA), Norplant, IUDs, and TR. All women were asked to complete their menstrual records for one year after delivery and report to the investigators monthly. The reference period method, recommended by WHO 1985[7,8] was used to analyze the vaginal bleeding records. The method adopted the woman as the unit of analysis, divided her diary into four consecutive 90-day periods i.e. period 1 (delivery to 90 days), period 2 (91 to 180 days), period 3 (181 to 270 days) and period 4 (271 to 360 days). Vaginal bleeding patterns were summarized within each period. The indices used to determine the vaginal bleeding pattern included number of bleeding/spotting episodes and number of bleeding/spotting days. Bleeding/spotting episode was defined as any set of one or more bleeding or spotting days bounded at each end by two or more consecutive bleeding-free days. Subgroups of subjects with clinically important bleeding patterns within each reference period were also analyzed. These subgroups included women experiencing 1) no bleeding throughout the reference period (amenorrhea), 2) prolonged bleeding, i.e. bleeding/spotting episodes lasting more than 14 days, 3) frequent bleeding, i.e. more than 5 bleeding/spotting episodes, 4) infrequent bleeding, i.e. 1 or 2 bleeding/spotting episodes, 5) irregular bleeding, i.e. 3 to 5 bleeding/spotting episodes and less than 3 bleeding/spotting-free intervals of 14 days or more, 6) none of the above (normal bleeding pattern). If prolonged bleeding occurred in conjunction with any one of infrequent, frequent of irregular bleeding, a woman was considered as having prolonged bleeding. No women failed to record bleeding/spotting episodes.

Since outcome variables (bleed-

ing/spotting episodes or bleeding/spotting days) were not normally distributed, the non-parametric Kruskal-Wallis test was used as a significant test.

Results

Three hundred and twenty two women were recruited and classified into four groups according to types of contraceptives they had chosen i.e. 114 DMPA, 99 Norplant, 32 IUDs, and 77 TR (Table 1). Table 2 shows baseline characteristics of the subjects. Most of them were labourers or farmers. The women in TR group were older and had higher parity than the other three groups. They also had lower percentage (2.6 %) of resumption of menstruation at 6 weeks postpartum but it was not statistically significant. Over half of them fed supplementary food to their babies early at the age of one month or younger.

Table 1 Sample size

Study groups	No.
DMPA	114
Norplant	99
IUDs	32
TR	77
Total	322

Table 2 Subject characterictics by methods of contraception

Characteristics	DMPA (%)	Norplant (%)	IUD (%)	TR (%)	p value
Occupation					
- labourers or farmers	82.5	74.7	72.8	75.8	0.0983**
Age (X ± SD)	24.3 ± 3.7	25.3 ± 4.1	24.6 ± 3.5	28.5 ± 3.2	0.0000*
Parity (median)	1	1	1	2	0.0000*
Resumption of menstruation at 6-week postpartum	12.3	12.1	9.4	2.6	0.1113**
Age of starting supplementary feedings					
< 1 month	10.5	6.1	9.4	11.7	
1 month	63.2	63.6	56.3	48.1	
≥ 2 months	26.3	30.3	34.4	40.3	0.3183**
Age of weaning					
3-6 months	4.4	3.0	6.3	2.6	
7-12 months	29.8	26.3	34.4	31.2	
> 12 months	65.8	70.7	59.4	66.2	0.8933**

* Significant
** Not significant

Number of bleeding/spotting episodes

Figure 1 shows number of bleeding/spotting episodes by study groups and reference periods. Generally almost all of our subjects had infrequent episodes of vaginal bleeding/spotting throughout their first postpartum year. The maximum episodes for all reference periods ranged from 2 to 8.

episodes for either group were 1,0,0 and 0, for the reference period 1,2,3 and 4 respectively. Median episodes among IUDs group increased from 1 in the first reference period to 3 in the fourth reference period. They had highest episodes for all reference periods when compared to other groups. Women in TR group resumed menstruation between 6 to 9 months after delivery, when they had a median epi-

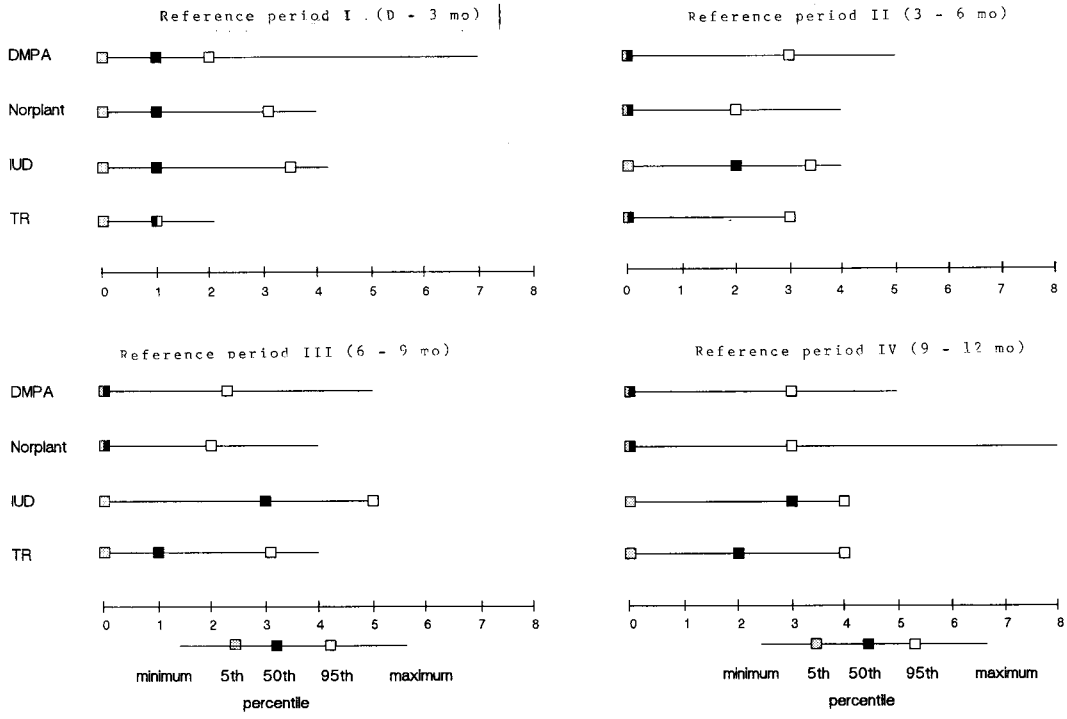

Fig. 1 Numbers of bleeding/spotting episodes in each 90-day reference period.

Overall, variability between women was small. The maximum range of 5th to 95th percentile was only 5 episodes. DMPA and Norplant groups had a similar pattern of bleeding/spotting in terms of episodes. The median values of bleeding/spotting

sode equal to 1. The median episode increased to 2 between 9 to 12 months after delivery.

Number of bleeding/spotting days

Number of bleeding and spot-

ting days is shown in Fig. 2. Variability of number of bleeding/spotting days between women was largest in reference period 1 (5th-95th percentiles = 0.0 - 34.5). Fifth to 95th percentiles of number of bleeding/spotting days in reference period 2,3 and 4 were 0-22.8, 0-20.8 and 0-20.4 respectively. Most women in DMPA and Norplant groups bled only during the first 90 days after delivery. There were more bleeding/spotting days among the DMPA group than either Norplant or IUDs groups in the first reference period.

Number of bleeding/spotting days in the IUDs group increased from 4 days in the first reference period to 11 days in the fourth reference period. There were no bleeding/spotting days, on average, among the TR group after delivery to 6 months postpartum. Vaginal bleeding resumed after 6 months post delivery and increased in number of days from 0.5 to 7.0 between 9 to 12 months .

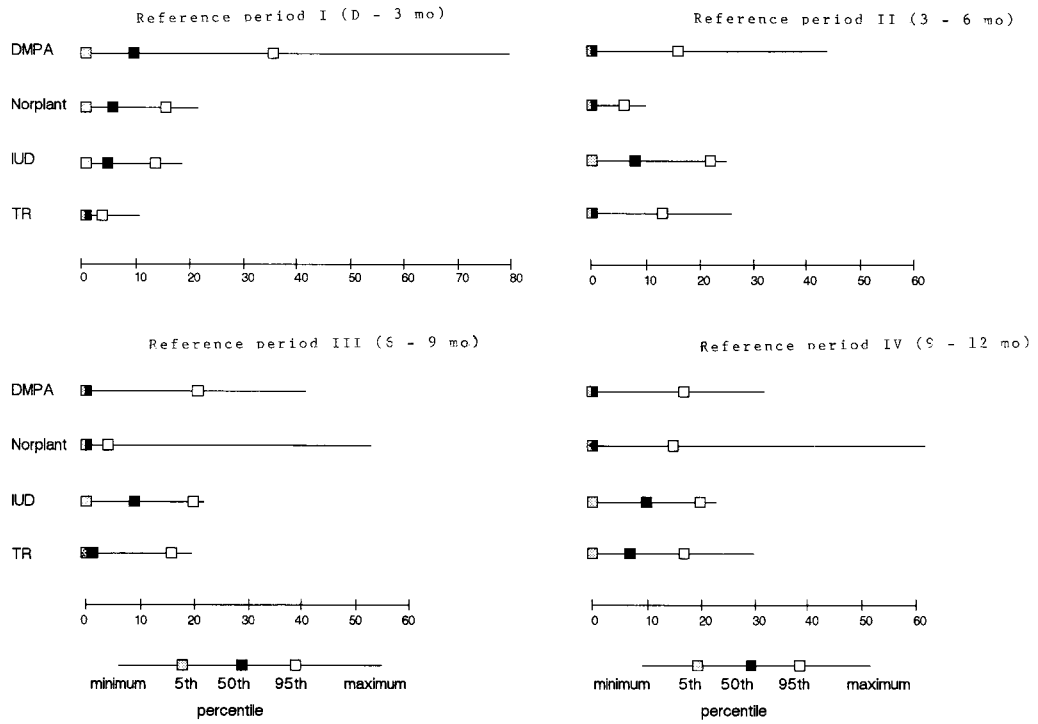

Fig. 2 Numbers of bleeding/spotting days in each 90-day reference period.

Bleeding pattern subgroups in each 90-day reference period

During the first three months after delivery (Fig. 3), the women in

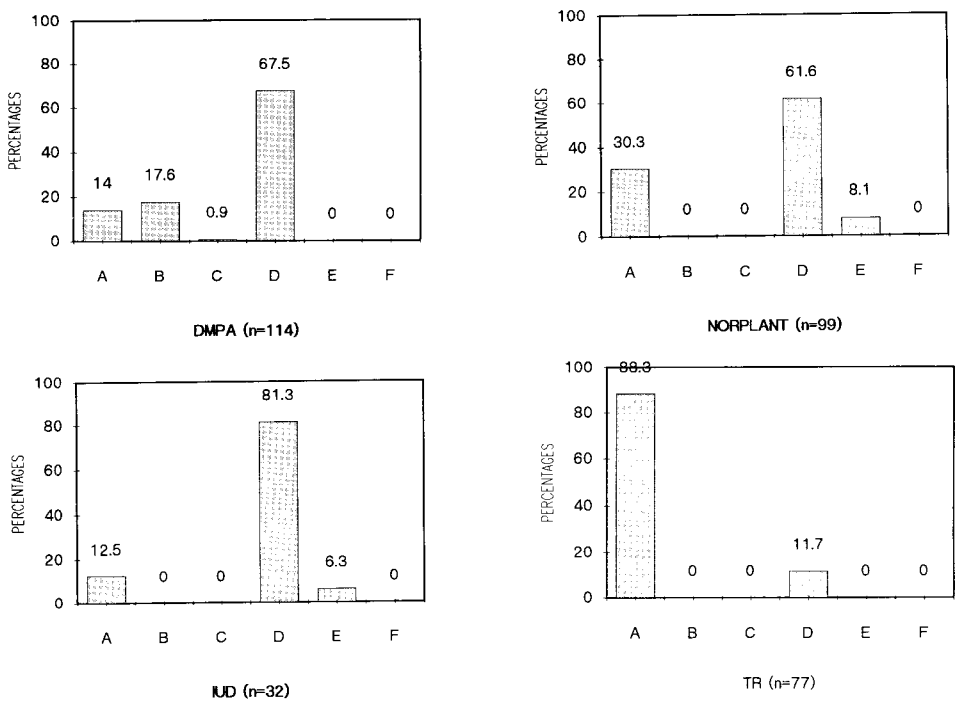

Fig. 3 Bleeding pattern subgroups in reference period 1 (delivery - 3 months).

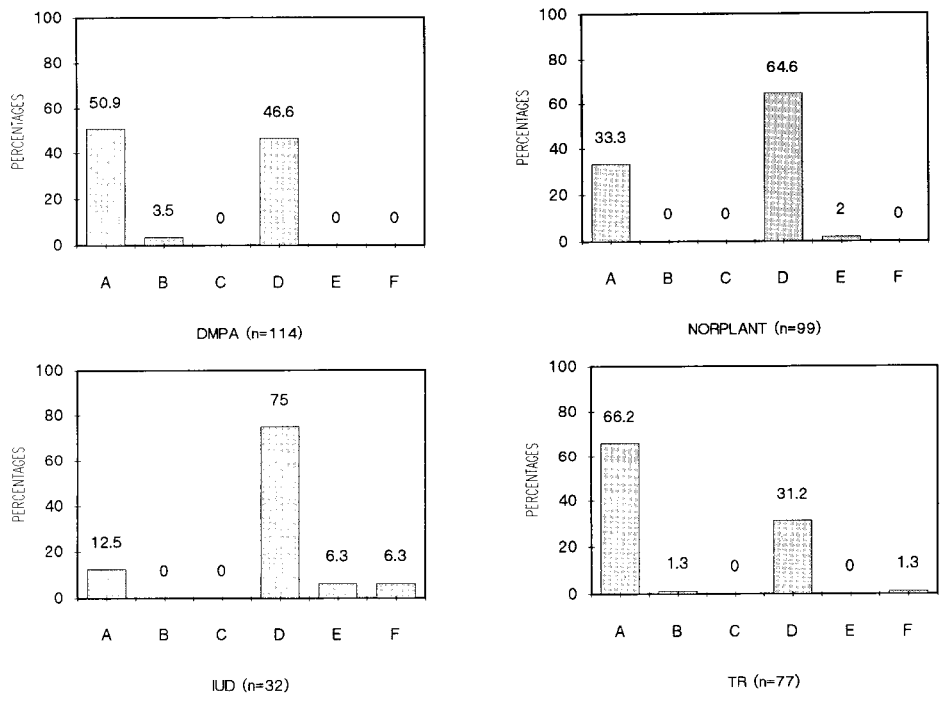

Fig. 4 Bleeding pattern subgroups in reference period 2 (3 - 6 months).

TR group experienced 88.3% amenorrhea, whereas, there were 12.5 % in IUDs group, 14.0 % in DMPA group and 30.3 % in the Norplant group. Bleeding pattern commonly occurred among study groups other than TR was infrequent. Prolonged bleeding occurred only in the DMPA group (17.6 %).

The proportion of women experiencing amenorrhea among the DMPA group rose progressively from 14.0 % in the reference period 1 (Fig. 3) to 71.1 % in the reference period 2 (Fig. 4). This was similar to the Norplant group where the figures were from 30.3 % during the first reference period (Fig. 3) to 81.8 % in the fourth reference period (Fig. 6). Contrary to the TR group, percentage of amenorrhea decreased steeply from 88.3 % in the first reference period (Fig. 3) to 27.3 % in the fourth period (Fig. 6).

Nearly 40 % among the TR group experienced infrequent bleeding throughout 3 to 12 months post delivery (Figs. 3-6). Normal bleeding occurred most frequently (43.8 %) among IUDs users during the fourth reference period (Fig. 6). Among TR

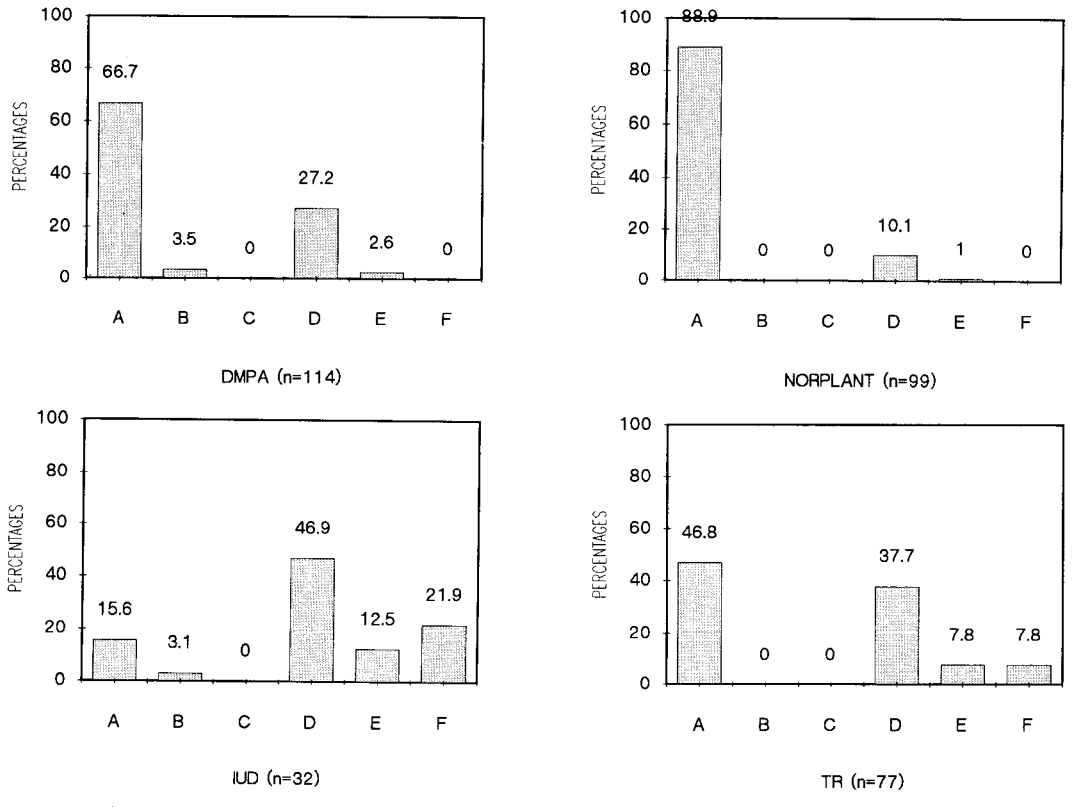

Fig. 5 Bleeding pattern subgroups in reference period 3 (6 - 9 months).

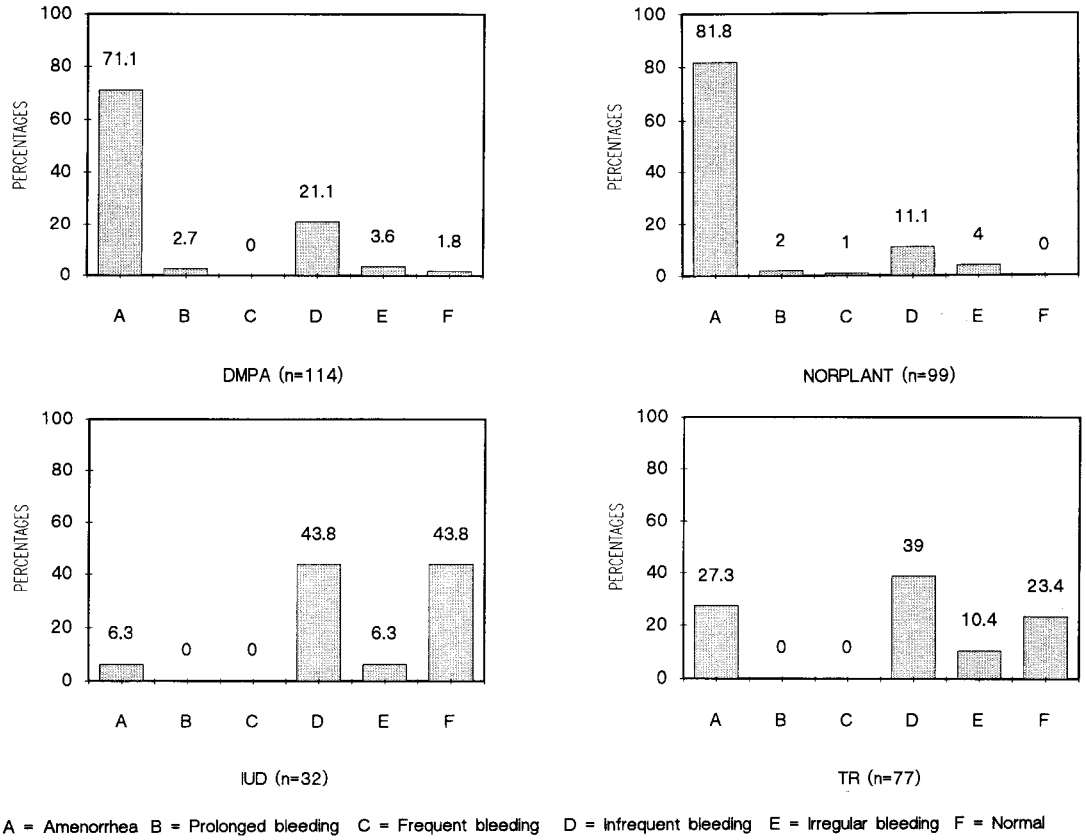

Fig. 6 Bleeding pattern subgroups in reference period 4 (9 - 12 months).

group, normal bleeding started to occur at 1.3 % between 3-6 months (Fig. 4) and increased to 7.8 % and 23.4 % between 6-9 months (Fig. 5) and 9-12 months (Fig. 6) respectively.

Discussion

Although women in the TR group were older and had higher parity than the other groups, the difference was not large or unlikely to influence vaginal bleeding patterns. Otherwise, study groups were comparable in terms of 6-week postpartum menstruation resumption, infant's age at onset of supplementary feeding and at weaning.

When compared to non-lactating women using the ovulation method from another study[5] who had, on average, 3 bleeding/spotting episodes and 15-16 bleeding/spotting days in each 90-day reference period, lactating women either using progestogen only contraceptives, IUDs or TR had much lower bleeding/spotting days and episodes. This was most likely due to the influence of lactation. Bleeding pattern in the TR group was comparable to lactating women who used no contraception. By the sixth postpartum month, about 20 to 50 % of breast feeding women using no

contraceptives were menstruating[9]. IUDs caused more bleeding/spotting days and/or episodes than progestogen only contraceptives or surgically sterilization. Contrary to tubectomized subjects, women using progestogen only contraceptives had vaginal bleeding early after the start of use and became amenorrheaic after three months postpartum.

Acknowledgement

Data used in this study was obtained from study project No.85915, entitled "Growth and health of infants whose mothers use progestogen-only contraceptives during lactation", which was partially supported by "Special Programme of Research, Development and Research Training in Human Reproduction", WHO.

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A Study of Squamous Cell Carcinoma of the Cervix in a Young versus an Older Thai Population

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Abstract: *A retrospective clinico-pathologic review was undertaken of all cases of invasive cervical squamous cell carcinoma accessioned at Siriraj Hospital from 1980 to 1985 in women 35 years or less and 55 years or greater. After study criteria were applied, 635 women were eligible for inclusion: 152 younger and 483 older. Younger women tended to present with early (Stage IB) as opposed to advanced (Stage III) stage disease found in older women ($p<0.001$). An earlier stage at presentation may be attributed to their higher frequency of contact bleeding (22% versus 2%). No shift in age distribution of disease to a younger population was found ($p>0.05$). A self addressed questionnaire was mailed to the patient's last known address in an attempt to increase follow-up data but significant deficits in the data base regarding time to first recurrence, its location, and date of death persisted. These deficits precluded an analysis of possible differences in tumour behaviour and virulence between younger and older women. Continued monitoring of this disease is warranted in view of world-wide reports of changing patterns of epidemiology and biology. (Thai J Obstet Gynaecol 1992; 4: 43-50.)*

Key words: cervical carcinoma, prognosis, age, epidemiology

Squamous cell carcinoma (SCC) is the major cause of death from cancer in the female population of Thailand[1]. This may be attributed in large part to the absence of an organized, country-wide cytology screening program for the detection and treatment of preinvasive cervical lesions commonly called cervical intraepithelial neoplasia (CIN).

Despite the presence of organized cervical cytology screening pro-

grams in some Western countries, reports are emerging from some of those countries of an increasing incidence of invasive SCC of the cervix in younger women[2-5]. Further, there are numerous reports from those nations documenting the emergence of an aggressive form of this cancer in young women[6-9]. In the young woman, invasive SCC of the cervix may be characterized by a poor response to conventional treatment with a short interval from diagnosis and treatment, to recurrence and to death[10-11].

The purpose of this study was to determine if a similar pattern of cervical SCC is emerging in the young Thai population. For this purpose certain characteristics of invasive SCC of the cervix in young women, 35 years or less at diagnosis, have been determined and compared to those of older women, 55 years or more at diagnosis.

Materials and Methods

The medical records at Siriraj Hospital from 1980 to 1985 were searched for all diagnoses of invasive SCC of the cervix involving women 35 years or less, or 55 years or greater. 1985 was chosen as a cut-off date to allow five years of follow-up. This involved a search of the records in the Cytology Unit, the Pathology Unit and the Statistical Unit of the Department of Obstetrics and Gynaecology. In addition the records of the Radiotherapy Division and the Hospital Statistics Record were searched. Follow up information was obtained from all those records as well as the Outpatient Department Gynaecologic Cancer Unit's records. In addition a letter requesting follow up information was mailed to the patient's last known address if she had not been seen in the pervious 12 months unless the date of death was recorded. A stamped return envelope was included with the follow up questionnaire[12].

From the above sources the following data were recorded as available: age, stage, presenting complaint, tumour size, treatment modality, time interval to recurrence and its location, and time interval to death.

All patients were staged according to FIGO criteria except firstly, routine intravenous pyelograms were not obtained. Secondly, on review of the histopathology, a 3mm level of invasion was used to separate microinvasive(Stage IA) from invasive (Stage IB or greater) SCC as recommended by the Society of Gynecologic Oncologists (SGO). This criterion also allows for better comparison with other published data from Western societies. All histopathology slides were reviewed by one of the authors (D.I.R.).

The only patients excluded from this study were those whose diagnosis and treatment were not established and/or completed in the study period, or those for whom the diagnosis of invasive (Stage IB or greater) SCC could not be confirmed on pathology review. Reasons for the

latter included the following:

1. the histologic diagnosis was made elsewhere, the patient being referred to Siriraj Hospital for radiotherapy or follow up.
2. the diagnostic slides were missing from the file.
3. transcription errors of name or record numbers occurred.
4. using SGO criteria, some patients were restaged as Stage O or IA.
5. on review the diagnosis was revised to verrucous carcinoma, malignant carcinoid tumour, oat cell (small cell) carcinoma, lymphoma or adenocarcinoma (one patient each per revised diagnosis).

For statistical test of significance, the *Chi square test* was used.

Results

Of the 1220 patients originally identified for this study, 635 remained eligible for inclusion after the application of study criteria. Figure 1 shows the number and percent of women in each age group diagnosed in each year of the study. No trend is apparent to suggest that the incidence of invasive cervical SCC is shifting to a younger age population ($p>0.05$). The stage at diagnosis for both groups of women for the entire study period is shown in Figure 2. The stage distribution at the time of diagnosis differed between younger and older women: younger women were more likely to present with early (Stage IB) disease while older women were more likely to present with advanced (Stage III) disease ($p<0.001$).

The commonest symptoms at presentation are shown in Figure 3. Only three of the younger, and seven of the older patients were asymptomatic. Five of the younger, and 12 of the older patients had no recorded symptoms. Many patients presented with multiple symptoms, the commonest combination being abnormal vaginal bleeding with leukorrhea. The percent of younger versus older women complaining of abnormal vaginal bleeding was almost identical (78% versus 77%). However, within the

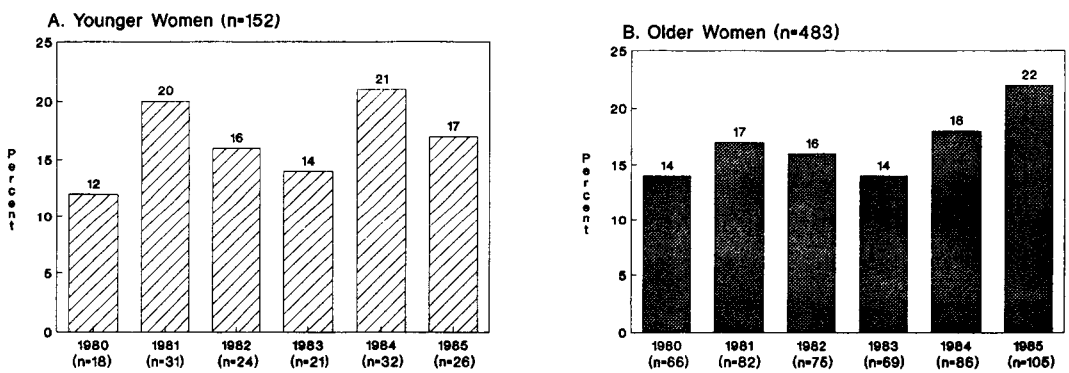

Fig. 1 Percent of women diagnosed by year.

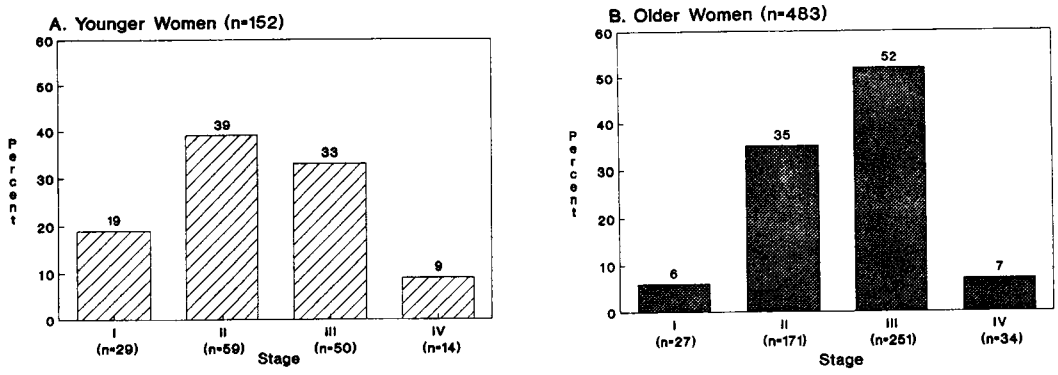

Fig. 2 Stage at diagnosis.

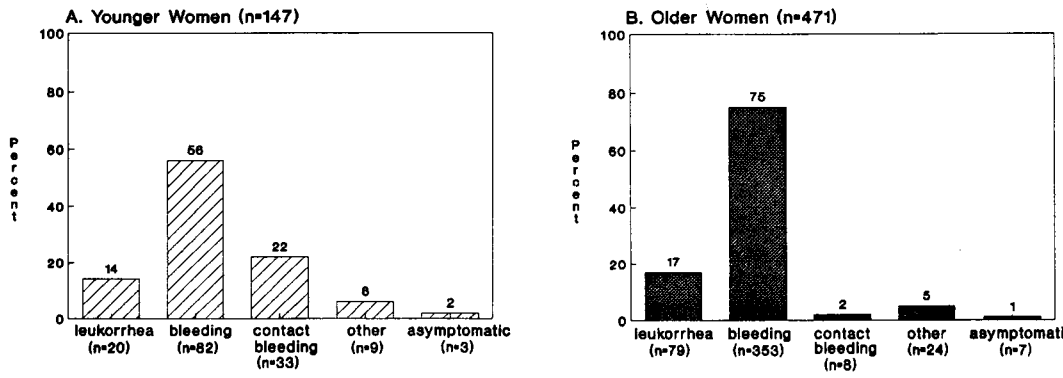

Fig. 3 Percent of women diagnosed by year.

category of abnormal vaginal bleeding, 22% of younger versus only 2% of older women complained of contact bleeding.

Other clinical or pathological features recorded during the review included lesion size, number of involved quadrants, tumour growth characteristics (exophytic, endophytic or ulcerative), presence of lymphatic or blood vessel invasion and depth of invasion. However, large amounts of missing data preclude comparison between age groups.

Some follow-up information was available on 556 patients (87.6%); the remaining 79 patients (12.4%) were not seen again at Siriraj Hospital after diagnosis (Table 1). Although the follow-up period ranged from 0 to 142 months, the majority of patients in both younger and older groups, were ultimately lost to follow-up. Follow-up to death was recorded (data for older women in parenthesis) in 0 of 29 Stage IB (4 of 27), 7 of 59 Stage II (26 of 171), 8 of 50 Stage III (51 of 251) and 4 of 14 Stage IV pa-

Table 1 Follw-up in months by stage

Stages	No.	Mean	Median	Range	No. with no Follow-Up
A. Younger women					
				14	
IB	29	70.7	62.0	0.5-136.0	4
II	59	47.2	26.0	1.0-129.0	6
III	50	31.1	11.0	1.0-121.0	1
IV	14	3.8	3.0	0.5-8.0	3
B. Older women					
				65	
IB	27	16.6	65.6	3.0-124.0	5
II	171	37.8	22.0	0.5-142.0	17
III	251	27.6	10.0	0.5-142.0	36
IV	34	21.4	8.0	0.5-133.0	7

tients (10 of 34). From the return of the questionnaire it was confirmed that an additional 5 younger and 18 older women were dead but no indication was given of the date or cause of death. The large amounts of missing data preculde a comparison of survival in each stage between younger and older women. Similarly, the absence of large amounts of data regarding time to recurrence and sites of recurrence prevent comparisons between groups of women.

Discussion

Although this is not a population based study, the analysis of data from Siriraj Hospital shows no trend to suggest that the incidence of invasive SCC of the cervix is shifting to a younger population. The ratio of younger to older patients remained relatively constant over the study period. The recently documented shift to a younger population reported from some Western countries is most likely related to changing female sexual habits with those women commencing sexual intercourse at a younger age and having multiple sex partners. The latter are well recognized major risk factors for the development of cervical cancer[13-14]. The absence of an apparent shift in this study suggests a more traditional and monogamous pattern of sexual behaviour exists for Thai women.

The analysis of data from Siriraj Hospital shows also that younger women tent to present with earlier stage disease than their older counterparts, a difference which was statistically significant ($p<0.001$). Why younger women tend to present earlier in the course of their disease is not entirely clear but may be related to the presenting symptom of contact

bleeding. If is can be assumed that older women are less likely to be sexually active than younger women, then contact bleeding from sexual intercourse might alert the younger women at an earlier stage to her disease. Thus, it is noteworthy that 22% of all younger women as opposed to only 2% of older women complained of contact bleeding.

It is also noteworthy that only three younger and seven older patients were asymptomatic at the time of presentation since a symptomatic presentation, especially in young women, correlates with recurrence and poor prognosis[11]. This may reflect with sampling bias of patients since Siriraj Hospital is a public hospital catering to the poor (as opposed to a private hospital) who have had limited access to health care. However, it almost certainly also reflects on the absence of a country-wide cervical cancer screening program which would increase the rate of detection of asymptomatic women.

This study emphasizes the necessity of a review of the original pathology specimens prior to the inclusion of any patient in a retrospective study. Of the original 1220 patients deemed eligible for inclusion, only 635 (52%) remained after pathology review. That exclusion rate is very similar to that reported recently from Canada where only 45 out of an original 83 (54%) patients remained eligible for inclusion[11]. Indeed, the exclusion rate as a direct result of pathology review from the Canadian Study was even greater since the first three reasons for exclusion in this study did not apply. Over the years diagnostic criteria are refined and/or changed. This is especially true in distinguishing Stage IA from IB cervical SCC. Some pathologists use the FIGO criterion of 5 mm invasion to separate the stages; others use the SGO's criterion of 3mm[15]. Still others do not recognize "microinvasive" as distinct from "invasive" disease. Since young women tent to present with early stage disease, the separation of Stage IA from IB disease is especially important. The inclusion of significant numbers of Stage IA tumours would tend to mask any difference in tumour virulence between younger and older patients. The importance of a standardized pathology review, especially in a retrospective study, has also been emphasized recently by Eifel and Hendrickson[16].

The large amounts of missing data preclude a valid statistical analysis of pathologic factors which might be indicative of a poor prognosis in both groups of women, or predictive of a difference in prognosis between younger and older women. Since invasive SCC of the cervix in older women presenting at Siriraj Hospital is preferentially treated by radiotherapy rather than radical surgery (as is the practice in many other hospitals), only small biopsies to confirm the diagnosis were available for review. Depth of invasion and the presence/absence of lymphvascular invasion cannot be assessed in such material.

Hysterectomies, which provide adequate specimens for such assessment, are only routinely performed in young women with early stage disease.

Similarly, a comparison of survival between younger and older women in each stage was not possible because of large amounts of missing data with respect to date of first recurrence, its location and the exact date of death. For example, in Stage IV disease which in almost all cases the patients might be expected to die of their tumour, no follow-up data was available for 3 of 14 younger and 7 of 34 older women and for only 4 of 14 younger and 10 of 34 older women was death known to have occurred. Although the response rate to our questionnaire was excellent[12], the replies were sometimes imprecise for they simply stated "dead", or "dead long ago". We have assumed in this study such patients died of tumour but especially for older women with early stage tumour with no documented evidence of recurrence, death from intercurrent disease could significantly alter the results.

Thus, the significant findings of this study are that younger women with cervical SCC tend to present at an earlier stage than their older counterparts. This is most likely the result of their noticing contact bleeding. Further, no shift of disease incidence to a younger population is apparent in this study. We are unable to determine if SCC of the cervix is becoming more virulent in the younger Thai patient as reported in some Western countries. However, a recent report form Japan[17], the first from an Asian country, confirms that age at diagnosis is also a prognostic factor in their study population. Continued monitoring of the incidence and biology of cervical SCC in Thailand would seem desirable.

Acknowledgements

The authors would like to thank Dr. Tunkae Yupraphat (Chiemprasert) through Dr. Oonjai Vaeusorn, Head of Fetal Pathology Unit, Siriraj Hospital, for financial support (grant no. D1072). In addition we would like to thank the following physicians for encouragement and assistance: Dr.Sommai Toongsuwan, Head of Division of Medical Statistics and Chairman of Department of Obstetrics and Gynaecology; Dr. Amorn Koetsawang, Head of Division of Gynaecological Pathology, Department of Obstetrics and Gynaecology; Dr. Visoot Vootiprux, Head of division of Radiotherapy, Department of Radiology; and Dr. Somporn Ekarat, Head of Medical Records and Statistics Department. Finally, we would like to thank the many technologists for their assistance.

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Endometrial Curettage in Nonmetastatic Gestational Trophoblastic Disease (NMTD)

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Abstract : *Endometrial curettage was done in 22 cases with NMTD from January 1979 to December 1986. The histology revealed no trophoblastic tissue in 13 cases, hydatidiform mole in 3 cases, trophoblastic tissue in one case and choriocarcinoma in 5 cases. One of 6 cases with abnormal uterine bleeding had negative curettage. Hysterectomy was done in 6 cases with complete families. All were histologically evident of disease. One case with negative curettage had choriocarcinoma in hysterectomied specimen. Because of the low yield of endometrial curettage, it was suggested only for patients with the symptom of abnormal uterine bleeding. (Thai J Obstet Gynaecol 1992;4: 51-53.)*

Key words : endometrial curettage, nonmetastatic gestational trophoblastic disease

Since the era of effective chemotherapy and sensitive hormonal tumour marker of malignant gestational trophoblastic disease, histopathologic diagnosis has become less important in the management of this disease[1,2]. The NMTD includes chorioadenoma destruens and choriocarcinoma confined to the uterus. The diagnosis is commonly based on an elevated human chorionic gonadotropin (hCG) titer and lack of extrauterine metastasis[3,4]. The benefit of histologic diagnosis is the starting of chemotherapy without delay[3]. Endometrial curettage is a possible mean to obtain tumour tissue for histological examination in NMTD. But it is not routinely done because the tumour is not commonly assessible to the curette and the danger of uterine perforation. This report reviewed our experience with histological findings of specimens in NMTD which endometrial curettage was done.

Materials and Methods

From January 1979 to December 1986, 22 (51.2%) of a total of 43 patients with NMTD underwent endometrial curettage before starting chemotherapy at the Department of Obstetrics and Gynaecology, Ramathibodi Hospital. All cases were placed in complete remission with 5-day

courses of Actinomycin D (10 microgram/kg/day). The mean (range) number of used courses was 3.9 (2-7). Hysterectomy was done during the first course of chemotherapy in 6 cases who desired no further pregnancy.

The medical records of these 22 patients were reviewed and presented descriptively.

Results

The mean (range) age and parity of the patients were 28.27 (21-47) years and 2.8 (0-9) respectively. All antecedent pregnancies were molar pregnancies except 2 cases following abortion and term delivery. The duration between antecedent pregnancy and curettage ranged from 1 to 6 months with the mean (SD) of 6.7 (13.0). There was no complication of the curettage.

The histological findings of endometrial curettage are presented in Table 1. No trophoblastic tissue was obtained in 13 cases (10 retained decidua, 1 late proliferative endometrium, 1 late secretory endometrium, 1 acute and chronic endometritis), hydatidiform mole in 3 cases, presence of trophoblastic cells in 1 case and choriocarcinoma in 5 cases. Only one of 6 cases, who had abnormal uterine bleeding during curettage, had negative histology.

The histology of hysterectomied specimens is presented in Table 2. The residual of trophoblastic cell was found in one case. Two cases with hydatidiform mole on curettage were chorioadenoma destruens. Three cases had choriocarcinoma even though negative curettage was found in one case.

Table 1 Histology of endometrial curettage

Histology	No
No trophoblastic tissue	13 *
Hydatidiform mole	3 *
Trophoblastic cells	1
Choriocarcinoma	5 #
Total	22

*1 case with uterine bleeding
#4 cases with uterine bleeding

Table 2 Histology of hysterectomied specimen

Histology	No
Trophoblastic cells	1 *
Chorioadenoma destruens	2 #
Choriocarcinoma	3 †
Total	6

Curettage histology :
* 1 trophoblastic cells
# 2 hydatidiform mole
† 2 choriocarcinoma, 1 no trophoblastic tissue

Discussion

In this series, more than half (13 of 22 cases) of the endometrial curettage revealed no trophoblastic tissue, as was the case in Berkowitz's report (20 of 37 cases)[5]. The explana-

tion is possibly that the tumour had already invaded into the myometrium or the technique of curettage might not have been done properly. However, this finding confirmed the low yield of this procedure. Fortunately, there was no uterine perforation or other complication in this series. But in 6 cases with abnormal uterine bleeding, only one case had negative curettage. This symptom highly suggested the possible residual disease in the endometrial cavity, and would be a useful guide as an indication of endometrial curettage.

According to the definition of NMTD, the disease is expected to be found in hysterectomied specimen. In this series, all 6 hysterectomied specimens were histologically evident of the disease. And the histological findings were well correlated with the curettage histology, except one case with negative curettage which had choriocarcinoma in the uterus. This case supported the previous discussion of unaccessible tumour by curettage.

Even though, pretreatment curettage has been advocated at the onset of chemotherapy for it provides prognostically important data regarding potential responsiveness to chemotherapy[5], the treatment of NMTD has been 100% successful regardless of the histopathology[3,6]. We would like to conclude that the pretreatment endometrial curettage was not necessary in the majority of our patients. It should be reserved for those with abnormal uterine bleeding.

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Actinomycosis Causing Pelvic Inflammatory Disease : A Case Report

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Abstract : *We report a case of severe pelvic inflammatory disease due to Actinomyces spp. in a 25 years old woman who recently had an intra-uterine contraceptive device removed. This disease may be seen more commonly if more such devices are inserted. (Thai J Obstet Gynaecol 1992;4: 55-58.)*

Key words : actinomycosis, pelvic inflammatory disease

Actinomycosis is a sub-acute suppurative and chronic granulomatosis disease usually caused by the anaerobic bacterium, *Actinomyces israelii* (rarely, other species of Actinomyces may be pathogenic)[1]. It usually produces disease in the cervicofacial ("Lumpy jaw") or ileocecal regions, the latter often associated with a history of appendicitis. Only rarely did it produce disease in the female genital tract. However, it is becoming an increasingly common cause of pelvic inflammatory disease especially in women who have used or are using an intra-uterine contraceptive device (IUCD).

We wish to report a case of Actinomycosis seen recently at Siriraj Hospital to draw attention to the association between use of an IUCD and pelvic inflammatory disease due to *Actinomyces* spp.

Case Report

A 25 years old married factory worker presented five months prior to admission complaining of dyspareunia and mild fatigue of one month duration. She asked to have her IUCD removed which had been inserted one

and one half years earlier, six months after delivery of her first child. She was seen in the Family Planning Clinic two weeks later for a prescription for birth control pills but also complained of daily vaginal spotting since the IUCD was removed. An adnexal mass was discovered at this time. She was seen in another clinic two weeks later complaining of abdominal discomfort for which a laxative was prescribed. By this time she had developed a low grade fever every evening.

One month prior to admission she came to Siriraj Hospital complaining of fainting episodes, increasing fatigue, urinary frequency and dysuria, and persistent abdominal discomfort. A diagnosis of a urinary tract infection was made for which antibiotics were prescribed. One week prior to admission she re-presented to Siriraj Hospital with the same complaints (minus those related to the urinary tract infection). At this time she could palpate an abdominal mass. She also complained of a 10 kg weight loss over the previous five months. Arrangements were made for admission.

At surgery multiple pelvic adhesions were present forming a large mass encasing the uterus, both adnexae, and part of the large bowel and omentum. After dissection the right ovary measured approximately 5 cm and the intra-operative impression was ovarian carcinoma. The cut surface of the ovary was soft, yellow-white, with focal areas of pus. The capsule appeared invaded. Multiple omental nodules about 1 cm diameter were present and it was also adherent to the peritoneum. 30 ml of sero-sanguineous ascitic fluid were present.

On microscopic examination the inflammatory process entirely destroyed the ovarian architecture but remnants of scarred Fallopian tube could be recognized. The inflammatory process consisted of multiple microabscesses which tended to coalesce. In the centre of many were characteristic clumps of "ray fungi" which had a reddish-purple colour on hematoxylin and eosin staining (Fig. 1). At the periphery of these clumps fine "rays" or "filaments" were seen extending briefly into the surrounding polymorphonuclear infiltrate (Fig. 2). Surrounding the abscesses were poorly developed fibrous connective tissue walls. This wall was most developed in the residual lamina propria of the Fallopian tube.

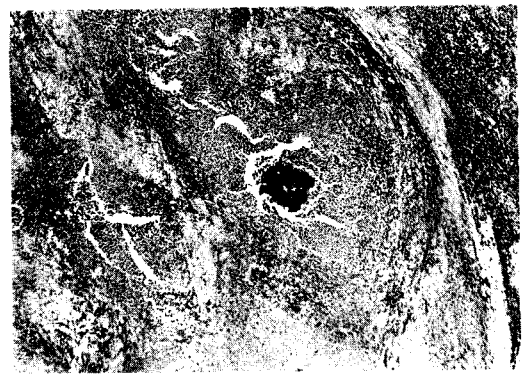

Fig. 1 Two microabscesses in the ovary, one of which contains a sulfur granule. H&E original magnification x 100.

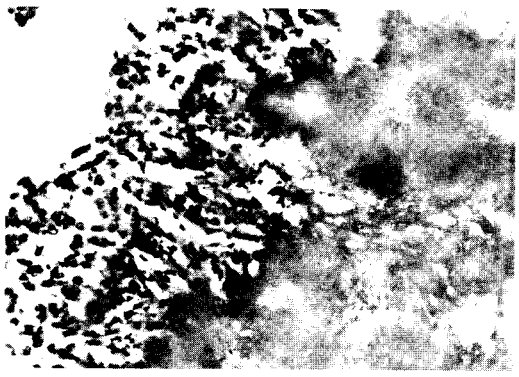

Fig. 2 The finely filamentous nature of the sulfur granule can be seen at its edge next to the rim of polymorphonuclear leukocytes. H&E original magnification x 400.

Discussion

Actinomyces spp. is a gram positive anaerobic bacterium which was previously erroneously classified as a fungus[1]. Since it is a filamentous bacterium, the descriptive term "ray fungus" has been applied. The bacteria tend to grow in solid clusters to form yellow clumps called "sulfur granules" which sometimes can be seen by the naked eye in the midst of the pus. It has a propensity of produce external sinus tracts surrounded by much necrosis and fibrosis.

Although historically the commonest sites of involvement have been the cervico-facial and ileocecal regions, virtually any tissue may be invaded. Only rarely, however, does it present in a disseminated bacteremic form. Whereas *Actinomyces* spp. infection of the female genital tract was rare it is becoming increasingly common especially in women using an IUCD. Colonization of the IUCD by *Actinomyces* spp. has been reported varying from 8%[2] to 14.5%[3] of cases. Over time, perineal contamination from a gastrointestinal source is thought to be the source of infection of the IUCD[1]. From the infected IUCD the organism spreads to involve the Fallopian tube then other surrounding structures. Monthly menstruation should inhibit the establishment of *Actinomyces* spp. in the endometrium.

There is still debate whether or not specific IUCD types are associated with higher infection rates than other types. Keebler et al[4] reported a higher infection rate for plastic than for copper IUCDs but Gulec and Gunalp[3] found no difference in infection rates by IUCD type. Similarly, some authors report that the infection rate increases with the duration of use of the IUCD[3] which is refected by others[5].

Actinomyces spp. are sensitive to both penicillin and tetracycline antibiotics which should form the basis of therapy[1]. However, since the organisms tend to grow in avascular tissues, prolonged high dose therapy may be required. Surgery may also be required depending upon the extent and location of disease especially to achieve drainage. If surgery is required the diagnosis of actinomyces should be suspected if necrotic tissue with a purulent exudate containing sulfur granules is encountered.

In summary, we report this case to alert gynaecologists to the association between severe pelvic inflammatory disease due to *Acti-*

nomyces spp. and the use of an IUCD. Further, the diagnosis of Actinomycosis should be suspected in any woman who has used an IUCD and who presents with signs and symptoms of subacute pelvic inflammatory disease.

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Paraurethral Vaginal Leiomyoma : A Case Report

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Abstract : *Vaginal leiomyoma is a rare solid tumour. The majority of these tumours occur in the midline of the anterior vaginal wall. The tumours are often asymptomatic but can cause dysuria, urinary frequency, urinary retention, dyspareunia and vaginal bleeding. This report describes a case of paraurethral vaginal leiomyoma. Simple excision is usually adequate treatment. (Thai J Obstet Gynaecol 1992;4: 59-62.)*

Key words : leiomyoma, vaginal tumour, female urethra

Paraurethral vaginal leiomyoma is a rare solid tumour and is often mistaken for cystocele, urethrocele, Skene's duct abscess, Gartner's duct cyst, urethral diverticula, vaginal cyst and vaginal malignancies. Since the first documented case was described in 1733 by Denys de Leyden[1] less than 250 cases have been recorded.

A case of asymptomatic paraurethral vaginal leiomyoma is presented. The clinical aspects, pathologic features and treatment of this rare vaginal tumour are reviewed.

Case Report

Mrs KH, a 39-year-old multiparous Chinese woman, reported with a history of gradually enlarging painless mass per vagina of 6 months duration. She was asymptomatic. Systemic examination was normal.

On physical examination, an apparently cystic, 4x3x3 cm mass was palpable arising in the suburethral region occupying the lower part of the anterior vaginal wall. The mass appeared to be continuous with the urethra. On palpation there was a partly cystic, partly solid mass 4x3x3 cm posterior to the urethral opening. The mass was fixed, circumscribed, nontender, non-compressible and with pressure no urethral discharge was obtained (Fig. 1). Speculum examination showed the cervix to be normal, with minimal mucoid discharge. Per

Fig. 1 Preoperative view. Typical suburethral, midline, anterior vaginal wall tumour.

vaginum, the uterus was normal size, mobile, anteverted and adnexae were normal. Urine analysis, complete blood count, ECG, chest X-ray were normal. Because of the uncertainty of what this mass represented, the patient elected to have surgical removal.

An excision was done through a small transverse vaginal incision. The oval circumscribed mass 4x3x3 cm was totally enucleated from the paraurethral structures with a urethral catheter in place. The wound was closed with absorbable sutures; the vagina was packed; and the urethral catheter was removed. The procedure was performed in the Out-patient Surgery Department. Her immediate postoperative course was unremarkable, and she was discharged 2 hours after the procedure.

Pathologic examination revealed a 4x3x3 cm, well-circumscribed mass that weighed 30 g. The mass was ovoid, firm and elastic in consistency and white. Sections through it showed pearly tissue, with the typical appearance of whorled bundles of smooth muscle fibers. The microscopic appearance of the mass consisted of anastomosed and whorled fascicles of fusiform cells of uniform size. The nuclei were elongated and mitoses were not frequent. Blood vessels were small and not numerous. Between the bundles of smooth muscle fibers were variable amounts of fibrous connective tissue (Fig. 2).

Discussion

Vaginal leiomyomas are exceedingly rare tumours that are often discovered as asymptomatic masses. It arises anywhere within the vagina, although most often on the anterior wall. The symptoms depend upon tumour size and location. If the tumours are near the urinary tract, associated symptoms such as frequency, urgency, dysuria and urinary retention may be present. This tumour may be any size from extremely small to large enough to occlude the vaginal canal. Vaginal leiomyomas are more com-

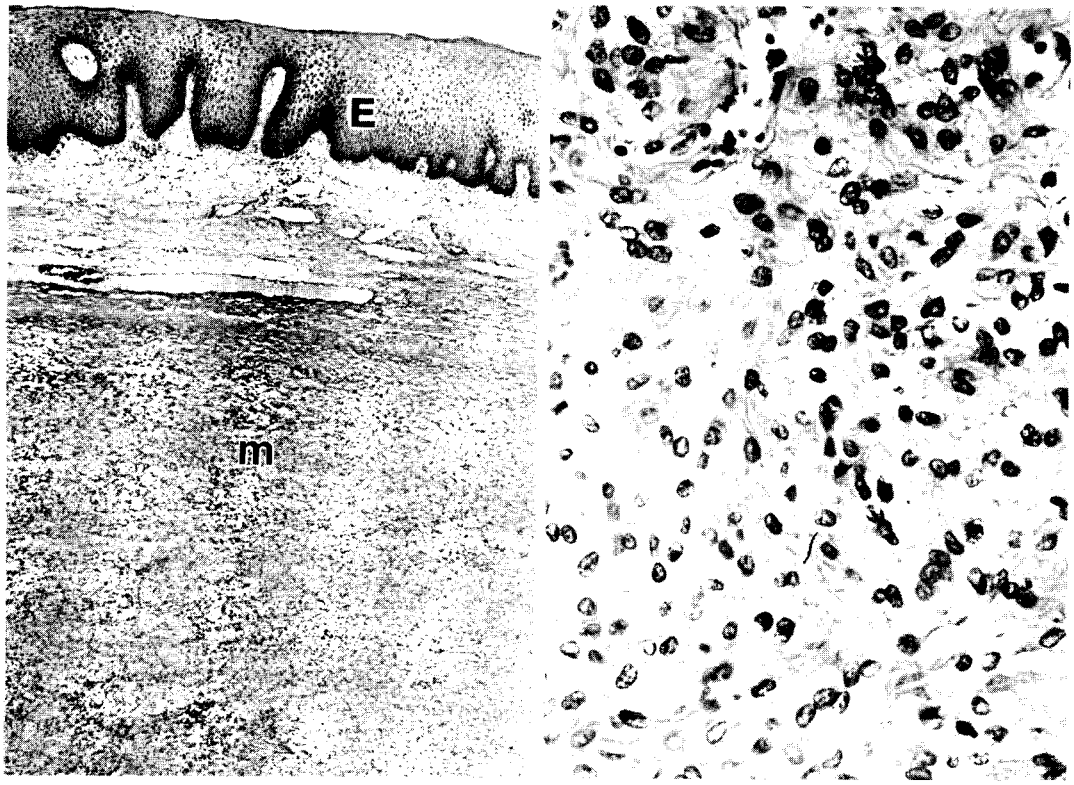

Fig. 2 Left : Low magnification reveals a well demarcated mass (m) which is located beneath the squamous epithelium (E). Right : Higher magnification of the mass reveals interlacing bundles of smooth muscle cells (H and E, x 10 and x 40).

mon in white women, whereas, the frequency of uterine leiomyoma in black women is relatively high[2].

Exact aetiology of development of this lesion is still not known although an endocrinal influence has been suspected[3]. Vaginal leiomyomas have been found associated with uterine leiomyomas, but there does not appear to be any correlation. On rare occasions, vaginal leiomyomas have been reported to be multiple and associated with leiomyomas of other organ systems[4].

The typical pattern of whorled bundles of smooth muscle fibers and fibrous tissue is homogeneous and gray-white in appearance. The vaginal mucosa is not involved, and the tumour can be separated easily from surrounding tissues. The same degenerative changes observed in leiomyoma elsewhere may also be seen, but rarely, leiomyosarcomas have been reported to arise from vaginal leiomyomas[5].

Simple excision is usually adequate treatment and can be done on an out-patient basis[6]. Caution should be exercised to prevent injury to the

urethra, bladder and rectum. A urethral catheter can help dissection and prevent urethral injury. Prognosis is excellent, only two cases of recurrence have been reported in the literatures[7]. When clinical findings are equivocal and differentiating one from another is impossible, histopathological examination of all paraurethral lesions excised would reveal further cases of this entity.

Acknowledgement

The authors wish to thank Assistant Professor Winyou Mitarnun of the Department of Pathology, Prince of Songkla University, for his pathological review and for taking the microphotographs.

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SPECIAL ARTICLE

IVF and New Technology

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Historical beginnings

Human IVF began in the late 1960s when Robert Edwards in Cambridge, England, first fertilized human eggs with human sperm in the laboratory[1]. Dr Edwards had made contact with a gynaecologist, Dr Patrick Steptoe, who had pioneered the use of laparoscopy in Britain, recognising that Steptoe's procedures allowed human eggs to be obtained from the ovaries. After that there were eight or nine years of frustration before the first pregnancy was achieved in 1976: the frustration was worse when that first pregnancy turned out to be an ectopic pregnancy[2]. The first baby, named Louise Brown, was not born from IVF until 1978[3], nine years after human IVF embryos had first been described, and this shows just how difficult the technology was to develop. For IVF to be successful every part of the clinical and laboratory procedure must be exactly correct; but on the other hand if everything *is* correct then we now know that it *will* work, and it will work immediately, and it will work predictably.

Australia was introduced to the milestones of IVF history in 1980. The fifth and sixth successful IVF pregnancies in the world were in Melbourne[4], and for the next year or two most of the world's IVF babies were Australian, as Professor Carl Wood and Dr Alan Trounson introduced controlled ovarian stimulation and timed follicle aspiration to clinical IVF procedures[5]. In 1983 Australia had the first pregnancy from transfer of an embryo that had been frozen and then thawed[6].

In 1984 a new technique was introduced which, for the first time in IVF technology, made use of the fallopian tube in women whose tubes are normal. The technique was developed in San Antonio by a colleague, Dr Ricardo Asch, with whom I had worked on the fallopian tube in 1980.

***Presented at the Annual Meeting of the Thai College of Obstetricians and Gynaecologists, Bangkok, October 25, 1991.**

Instead of leaving egg and sperm in the laboratory he transferred the eggs and prepared sperm into the fallopian tube at the same laparoscopic or mini-laparotomy procedure that had been used to obtain the eggs[7]. Immediately there were pregnancies. His daughter helped him name the revolutionary technique "gamete intrafallopian transfer", or GIFT. Around the world today IVF programs, good and not-so-good ones, consistently show better pregnancy rates with GIFT than with IVF and uterine embryo transfer.

However, in the same year, 1984, a major change was taking place in IVF. Pierre Dellenbach, working in France, showed that follicles could be aspirated and eggs obtained through the mucosa of the vaginal vault, guided by ultrasound[8]. Even more importantly, Dellenbach showed that general anaesthesia was not required for egg retrieval. Around the world, by 1986, numerous ambulatory care IVF programs had been established, requiring no hospital admission. Our own first program, at Royal Prince Alfred Hospital, began in February 1986 and there were pregnancies in the first month of operation. At the same time we also began laparoscopic GIFT at Sydney IVF, also with pregnancies straight away[9]. The laboratory technology that made these immediate results possible was that of the very well established IVF program at the Royal Women's Hospital in Melbourne, and shows the importance of correct introduction of new technology.

In 1987 a variation of GIFT was introduced. Known by such names as "zygote intrafallopian transfer" (ZIFT)[10], "pronuclear-stage transfer" (PROST)[11], "tubal embryo-stage transfer" (TEST)[11] and "laparoscopic fallopian embryo transfer" (LAP-FET), these procedures meant that, instead of unfertilized eggs and sperm, fertilized eggs or pre-embryos were successfully transferred to the tube. This technique was - and is still - especially valuable for the treatment of couples where there is oligospermia: it combines the advantages of GIFT (development of the early embryo in the tube and correct transport into the uterine cavity) with the advantages of IVF (confirmation of fertilization before transfer). In 1987 we introduced, as the Chairman kindly pointed out, the technique of catheterizing the fallopian tube from the vagina[12], making "ultrasound" or "transcervical" GIFT possible.

In the most recent breakthrough, in 1988 in Singapore, the world's first pregnancy occurred from sperm microinjection for severe male infertility[13]. In this technique individual sperm cells are placed in the perivitelline space between the zona pellucida and the egg cell membrane. We reported normal karyotypes among embryos produced this way[14] and went on to produce with we think would have been the world's second and third babies from sperm microinjection[15]. I will elaborate on sperm microinjection below.

This, then, is an introduction to

the time-frame in which technology has been introduced to IVF so far. It gives a preliminary idea of how new technology has evolved and how existing technology can be put to new use.

Physiological considerations

In normal reproduction, sperm move from the vagina through the cervix, through the uterus and fertilized the egg in the fallopian tube[16]. The egg has been ovulated from the ovary and will reach the ampullary isthmic junction in about 30 minutes. The egg will be fertilizable for about twelve hours. The sperm in the tube are very few, may be not more than five or ten-a contrast to the millions ejaculated at the start, and the hundreds of thousands that reach the uterus. Why are so few sperm needed in the tube naturally when thousands must be placed in the tube for GIFT to work ? If only we knew !

The fertilized egg stays at the ampullary-isthmic junction for another two or three days before travelling down the tube to the uterus. While still in the tube the fertilized egg develops to the stage of a morula, a solid ball of cells. As it enters the uterus it will become a blastocyst.

With in-vitro fertilization we cannot yet achieve this. Embryos will not divide as quickly as this with present culture technology. Instead with IVF we know from experience that if culture technology in an IVF program is good then we have the best results if we transfer in two days or three days: usually the embryo is only at the stage of two cells or four cells at the time of the transfer. When laboratory conditions and technically not perfect then better results come from transferring on day 1, at the pronuclear stage, than on day 2 or 3[17], although the pregnancy rates will be significantly lower than day 2 or 3 transfers in better laboratories. For good IVF laboratories too-but especially in not-so-good IVF laboratories-the difficulty is that the embryo does not grow as fast in the laboratory as it does in nature, and the longer we keep the embryo in the laboratory the more behind the embryo gets.

While IVF embryos lag behind in development, this is not the case for the uterus. The sequential action of progesterone on the endometrium is well known to all gynaecologists. If you look at the endometrium on day 16 or 17, when we should be transferring the embryo, the endometrium will normally be developing the first histological signs of secretion, namely basal vacuolation. But in stimulated IVF cycles we have found on endometrial biopsy at the time of embryo transfer that the endometrium is advanced: the stroma may look like day 21, the endometrial glands may look about day 18 or 19 (Jansen RPS, Anderson JC, and Russell P, unpublished information). The endometrium is both abnormal and advanced. So with IVF on the one hand we have the endometrium which is developing too quickly, there is too much progesterone effect. On the

other hand we have the embryo which is slow . Embryo transfer studies in other mammals, in which synchronous and asynchronous transfers are compared, show that the embryo can wait, if the endometrium is behind, but if it is the embryo that is behind and the endometrium is ahead then the embryo often cannot catch up. This is the single most important limitation on IVF today. For IVF to work acceptable we need to stimulate the ovary to bring more that one follicle to yield mature eggs, but when we stimulate the ovaries we get an endometrium which behaves as if there is too much progesterone, it develops too far too fast, and the embryos cannot often catch up, which is probably why only a small proportion of IVF embryos result in viable fetuses, even with the best of present technology.

Present culture techniques will not bring embryos to the blastocyst stage on time. Attempts have been made to improve embryo development in vitro by trying to duplicate what the tube can do with epithelial co-cultures[18], but improvement is so far only marginal.

A second difficulty with IVF is the mechanical difficulty, sometimes, of transferring embryos through the cervix and its mucus. We have found that it does help to use ultrasound to see the catheter in the cavity of the uterus for accurate transfers, but still there may be translocation of the embryos away from the deposition site as the catheter is withdrawn.

The advantages of utilising the tube for GIFT, ZIFT, PROST and so on are therefore several. First, laparoscopic deposition of gametes or embryos through the fimbrial end of the tube to the AIJ is mechanically more reliable than transfers to the uterus. Second, early embryo development in the tube is likely to be normal instead of slow (although the endometrium is still relatively advanced). Third, entry of embryos into the uterus from the tubes is more correct than introduction through the cervix.

The trouble since 1985 has been, however, that, just as laparoscopic tubal transfers were being shown to have important advantages over uterine embryo transfers, IVF itself had moved away from laparoscopy to a walk-in, walk-out outpatient basis utilizing transvaginal ultrasound, with the patient remaining awake for the procedure. So Dr John Anderson and I developed a catheter system in order to transfer eggs or embryos into the fallopian tube by ultrasound.

Ultrasound-guided transvaginal catheterization of the tube

The KJITS-2000 catheter system (Figure 1) consists of (a) an outer opaque Teflon canula, which is placed by ultrasound control (or slightly less reliably by tactile sensation alone) [REF] at the lateral angle of the endometrial cavity, and (b) an inner clear Teflon tubal catheter, of external diameter 0.6 mm. We reported the catheterization technique in 1987[12],

pregnancies from artificial insemination using the catheter in 1988[19], and the world's first pregnancy from embryo transfer through the catheter in 1988[20]. Our general experience has been that the chance of pregnacy with ultrasound-GIFT or ultrasound-fallopian embryo transfer (ultrasound-FET) is, with current techniques, about two-thirds that which we get with laparoscopic cannulation of the tubes from the fimbrial end.

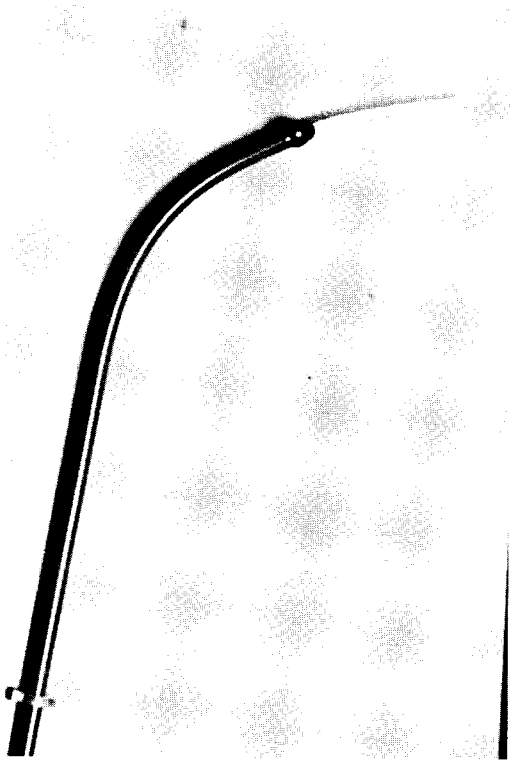

Fig. 1 KJITS-2000 fallopian tube catheter set (William Cook Australia Pty Ltd., Brisbane Technology Park, Eight Mile Plains, Queensland 4113, Australia) consisting of an outer bulb-tipped cannula, which reaches the lateral angle of the endometrial cavity, and an inner fine catheter, which passes through the uterotubal junction into the fallopian tube.

Ovarian stimulation

The more embryos that are transferred the higher the pregnancy rate. That means that for IVF and GIFT procedures to have a good chance of success in practice we should stimulate the ovaries. In stimulating the ovaries with human menopausal gonadotropin (hMG) injections we do what we can to prevent the LH surge. In principle, we can use hMG alone, hMG with clomiphene, or hMG with GnRH analogs: with all but the last of these regimens spontaneous LH surges compete with exogenous human chorionic gonadotropin (hCG) injections to initiate ovulation.

Clomiphene increases pituitary FSH release and has several advantages. One is that it is cheap. Second, because it is an anti-estrogen, it holds the endometrium back a bit and that is probably good for IVF. The disadvantages are (a) that it does not prevent the endogenous LH surge, so even with careful monitoring some stimulation cycles will be spoiled by unscheduled ovulation, and (b) that the retarded endometrium is qualitatively abnormal, increasing the chance of faulty implantation and spontaneous abortion.

GnRH analogs such as leuprorelin (or leuprolide in the United States) inhibit pituitary gonadotropin secretion and thus prevent both endogenous LH surges and clomiphene-induced abnormalities in the endometrium. When we introduced leuprorelin at Sydney IVF we notice an overall

improvement in our GIFT pregnancy rates[9], even though we reduced the number of eggs from four down to three (Figure 2). We also noticed that the chance of miscarriage was less than on clomiphene.

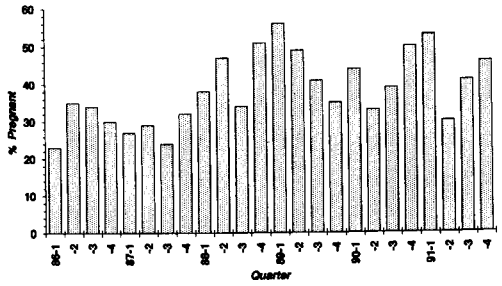

Fig. 2 Pregnancy rates by quarter for laparoscopic GIFT at Sydney IVF since start up in 1986. The introduction of leuprorelin instead of clomiphene in association with hMG in the fourth quarter of 1988 maintained the pregnancy rate despite a reduction from 4 to 3 in the number of eggs transferred.

Female limits to IVF: Egg or embryo storage

The more eggs we get the better the chance of pregnancy. But even if we transfer only three or four eggs we still see that the more eggs we get the better the chance of pregnancy. So it is not just the number of eggs we transfer but the more eggs we get the better the chance of pregnancy - even though we just transfer a maximum of three eggs. Why is this ? The reason is that a woman, when she is a 20 weeks fetus, has up to about eight million eggs, all contained in primordial follicles, after which there is a steady decline in number of eggs in the ovaries; she produces no new eggs; the eggs that are there get older and older; there is always a proportion of the follicles starting to develop, but until puberty and the commencement of ovulatory cycles all of these follicles undergo atresia and the eggs are lost. By the time she is born she has only one and a half million eggs. By the time she is a teenager she has about 300000 eggs. The younger a woman is the more follicles will be starting to grow spontaneously at any particular time and hence the more follicles will develop with hMG. So what we are seeing is that it is younger ovaries that do better with GIFT and IVF - and younger ovaries produce younger (and more) eggs, which in turn are more likely to lead to pregnancy than older eggs. So the number of eggs we get on stimulation depends more on the youth of the ovaries than on the dose of hMG. If we keep the number of eggs or embryos transferred constant then the younger the eggs the better the results.

The age of a woman (and the age of her eggs) defines the present limits of technology in treating women: the clock cannot be turned back ! How do we help in the future ?

The answer will probably be to obtain eggs at a young age and store them. With existing technology this is possible only with ovarian stimulation, egg recovery, IVF and then embryo cryostorage. But what about unmarried women ? This is almost science fic-

tion now but it is likely that in the future those wealthy women who can afford it will be able to choose to keep some immature eggs frozen from the age of 25 for 10 or 15 years, until they want to get pregnant. The eggs may need to be at the germinal vesicle stage to resist freezing and thawing without damage.

Male limits to IVF: Sperm microinjection

The limitation of IVF for men is overcoming the very low sperm count. How can new technology in IVF help the man with the low sperm count ?

Figure 3 shows fertilization with GIFT, standard IVF and IVF by sperm microinjection at Sydney IVF. With GIFT we do not get pregnancy with fewer than about 50000 motile sperm per drop (30 ul); with IVF, we do not get fertilization with fewer than about 8000 motile sperm pre drop; but with sperm microinjection into the perivitelline space (Figure 4) fertillization is possible with much lower sperm counts after sperm preparation.

As mentioned above, the first pregnancy from sperm microinjection took place in Singapore[13]. Sydney IVF's first two pregnancies, in 1990, were among the next to occur[15]. We now have more than 10 successful pregnancies and the pregnancy rate in 1991 was 11.9% of initiated cycles, all among couples with desparately low sperm counts and previous unsuccessful conventional IVF. But the fertilization rate with microinjection is still relatively low at about 15 to 25% of eggs fertilized. We need to ask why this is. Where are the technological difficulties at the moment with sperm microinjection ?

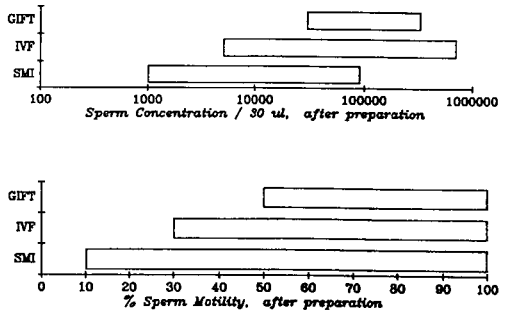

Fig. 3 Sperm concentrations per 30 ul and motility of prepared semen samples associated with fertilization at GIFT, conventional IVF and IVF by sperm microinjection at Sydney IVF.

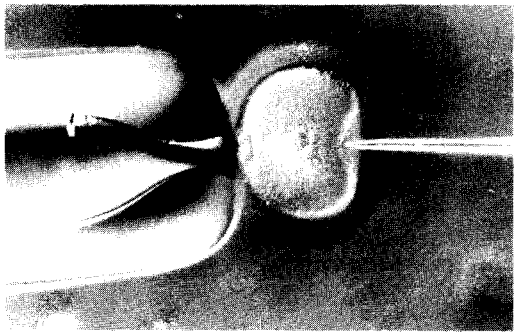

Fig. 4 Sperm microinjection below the zona pellucida into the perivitellin space. Photographed by Janine Lippi, Sydney IVF.

Firstly, we were worried that by collecting just one sperm and putting it next to the egg we were forgetting Nature's role in sperm selection. So ethically, in introducing this new

technology, we had to do experiments on the embryos before we could begin clinical procedures. This required approval from the Ethics Review Committee of the Central Sydney Area Health Board-the first time in Australia that and institutional ethics committee had given explicit approval for embryos to be created *solely* for the purpose of research. We compared the results of sperm microinjection with karyotypes of embryos on conventional IVF and we found that the abnormality rate was only 22% with fertilized injection and 30% for ordinary IVF[14]. So we and our institutional ethics committee were happy with that and we proceeded then with the clinical studies.

Secondly, sperm during transit of the male and then the female reproductive tract are protected by an acrosome. Once capacitated, sperm are capable of undergoing the acrosome reaction - an event the normally occurs upon binding of the sperm to the zona pellucida. Excretion of the acrosome releases enzymes that digest a path through the zona. More importantly, here, is that the loss of the acrosome reveals binding sites on the inner acrosomal membrane that allow the sperm to bind to the egg cell plasma membrane - the first step directly leading to fertilization of the egg. When we do microinjection we inject the sperm through the zona, and the sperm generally will still have the acrosome: they will therefore not bind to the egg. By manipulating culture conditions we can increase moderately the proportion of sperm in a prepared sperm sample that have undergone the acrosome reaction spontaneously[21], but because the proportion of sperm naturally acrosome reacted but still healthy is low the fertilization rate with sperm microinjection is also low.

We do not yet have a solution to this problem of injecting only acrosome-reacted sperm, but Sydney IVF and other research groups are looking at several possibilities, including application of a high voltage electric current on the sperm, called electroporation or electrofusion. This is like applying an electric shock to the sperm to lose the acrosome reaction. We do get some success with increased acrosome reaction but at the same time sperm motility falls very briskly, so that this is one of the technological frontiers in this area of sperm microinjection.

Clinical indications for assisted conception

When should we introduce technology to getting pregnant for individual couples ? Getting pregnant is sometimes easy and sometimes difficult. To decide if IVF or GIFT or an assisted conception procedure is the right decision for infertile couples we need to consider the mathematics - in particular we need to consider the statistic known as *fecundability*, the monthly chance of getting pregnant. Like everything in nature, its value has a distribution across the population. The average is about 20% per

month, or as a proportion, 0.2. But the extremes range from about 3% per month to about 50 or 60% per month. At the low end of this range the chance of pregnancy, even though "normal", may only be 20% per year, so many of these normal couples will see the doctor with infertility. On the other hand there are other people who get pregnant very easily and will still be fertile when there are one or two infertility factors present.

When we investigate infertility we may or may not come up with a diagnosis. If there is azoospermia, or anovulation, or genital tract occlusion, we have a reason for complete infertility - for sterility. Otherwise we have a situation of relative infertility. Choices of treatment for sterility or for infertility then consist in principle of (1) an attempt at cure (e.g. an operation for fallopian tube obstruction), or (2) to resort to assisted conception. IVF was invented to overcome irreparable fallopian tube disease, but from the beginning it also had the advantage that it can overcome many different causes of relative or relative infertility at once - e.g. by overcoming a low sperm count, in overcoming endometriosis, in overcoming peritubal adhesions, in overcoming cervical factors. This means that IVF and assisted conception may be indicated in many more cases than were originally intended. It is especially useful when there are several things wrong at once (Table I). If you have some decrease in sperm count, some endometriosis, some peritubal adhesions, and you do GIFT then the chance of pregnancy is just as good as it is in any one - or none - of them.

So the question of whether or not to use assisted conception to help a couple get pregnant does not depend so much on the exact diagnosis. Instead, it depends on *time:* first, on the amount of time the couple has spent

Table 1 Effect of one or more theoretical infertility factors on the monthly and yearly chance of natural conception, and on the average expected time to achieve concpetion. Assisted conception procedures often have an equal chance of working irrespective of whether such infertility procedures are present or not.

No. infertility factors*	Monthly probability	Yearly probability	Av. time to conception
0	20 %	94 %	4 months
1	4 %	40 %	2 years
2	1 %	10 %	7 years
3	0.2 %	2 %	40 years

* each factor, such as endometriosis, oligospermia, peritubal adhesions, cervical disease, polycystic ovary syndrome etc. is assumed to be of such severity that it decreases the monthly chance of conception to one fifth of what it would otherwise be.

in trying to achieve pregnancy (the duration of the infertility); and second on the amount of time that is left to get pregnant (how old the woman is, and the urgency of getting pregnant). Once a decision is made to use assisted conception the question becomes, What form of assisted conception is best? The answer here is to make use of the tube if it can be shown to be normal (e.g. GIFT) and to use IVF instead of GIFT if (a) there is doubt about sperm function (the embryos can still be transferred to the tube, e.g. ZIFT, PROST, FET), or, of course, (b) if the fallopian tubes are missing or are damaged (when uterine embryo transfer is the only option).

Now, if assisted conception is cost effective in a rich country like America then it is in principle cost effective everywhere. In every country where infertility is important you must use those methods of getting pregnant that are most effective, and if it is cost effective to do IVF in one country where costs are high, it can also be cost effective in another country where many of the costs are much lower. A developing country has even scarcer resources to waste on ineffective treatment that a rich country has. Once the capital equipment for an IVF program has been bought then the item that most determines the cost of providing assisted conception services is the price charged for hMG (for Humegon from Organon and for Pergonal from Serono), which are about the same real price in every country in the world, and so are relatively much more expensive in poorer countries than in rich countries. The costs of producing hMG are set to fall soon as both companies adopt gene technology (recombinat DNA technology) to synthesize FSH and LH instead of having to extract hMG from the urine of postmenopausal woman. More than any other single factor, the availability of accurate and effective assisted conception will depend on the pricing policies these companies adopt for their product in areas of the world that are not yet rich.

Introducing assisted conception technology

Because IVF and related assisted conception procedures are almost always expensive for patients you would think that great care and other peoples' experience would be important when new programs are started. Unfortunately, this is not always so. Because infertile couples are so desparate that they often will pay for assisted conception when those who are providing it have not yet brought themselves up to the high standard that is required in every single aspect of the IVF process before pregnancy becomes likely. The cost of the doctors' and scientists' education is paid for by the patients, who can least afford it. But this slow and cruel development of IVF is becoming harder and harder for develpoing IVF programs to follow, because the patients in poorer countries who can afford IVF can now

usually also afford to travel to a centre where IVF practices are already well developed. It is therefore essential that a city like Bangkok should develop an IVF program that is immediately good enough for rich patients form Bangkok to stop travelling elsewhere for treatment. Unless the patients have the confidence to stay in Bangkok, the doctors and scientists and nurses involved with IVF in Bangkok will not have the confidence and experience to improve IVF facilties for the poorer citizens who cannot readily afford to travel, for example, to Singapore for treatment.

It is important to realise, first, that, outside Riyadh in Saudi Arabia, government funding alone has not been enough anywhere for a successful IVF program to be established - not in Britain, not in Australia, and not in the United States, so it is unlikely that you will ever find public funds sufficient to develop IVF properly in Bangkok. In Cambridge it was a private venture at Bourn Hall by Steptoe and Edwards that popularised IVF (although they built a large private hospital just as IVF became an outpatient procedure, and the venture was apparently sold to the Serono company to enable it to stay in business). In Melbourne, IVF developments at the universities were always supplemented by the fees earned from private IVF practice. Sydney IVF is a private IVF program that works in close association with a public hospital, Royal Prince Alfred Hospital, to make IVF available to people who cannot afford private IVF. Around the world, all the major developments have taken place with the co-operation and the cash flow generated by the private side of IVF, so that patients who are the wealthiest are the first to be treated, but they give the IVF program the momentum to develop. Most of the important research has been done in this joint environment between the private side of IVF and the public hospital side.

After developing ultrasound-based outpatient GIFT and FET procedures and after achieving success with sperm microinjection, Sydney IVF in 1989 began its overseas consultancies. Our experience in the commencement of two IVF programs in Sydney, one IVF program in Jeddah, Saudi Arabia, one IVF program in Kota, India, and numerous short consultancies to troubleshoot IVF programs with problems elsewhere in the world leads to some interesting conclusions on how to introduce IVF technology to an existing infertility treatment program.

In each of the IVF programs we have established, pregnancies were achieved in the first month of operation, so we can safely conclude that techniques that work are fully transportable. We have also developed over the past year or two ways of analyzing why other programs have difficulties, why pregnancies don't happen.

Usually the equipment is perfect. When we travel we see the same equipment everywhere, whether IVF programs have good or bad results. The space in the lab is usually not a

problem: some of the most successful IVF laboratories are very small. Instead it is the way things are done that is usually the problem - the way in which the egg and embryo are looked after is in principle difficult and is in practice often wrong. For the unfertilized egg, protected chemically by the buffering capacity of the cumulus mass, temperature is absolutely critical: 10 minutes at room temperature is enough to destroy the spindles necessary for correct later development[22]. For the cumulus-free embryo, temperature is still important but now the chemical enviroment, including pH and the availability of the correct nutrients and the absence or toxins that can easily diffuse accross the zona are important. Figure 5 shows the micromonitoring equipment Sydney IVF has developed to measure temperature, pH, pCO_2 and relative humidity of the microenvironments into which eggs and embryos are put.

The macro - and micro - environment must be very carefully controlled. Figure 6 shows the temperature in a well-controlled incubator; the incubator is correctly set at 37.5 and manipulations to it makes no difference to the internal temperature where the eggs and embryos are kept; this is a good situation. Figure 7 is an example from a consultancy that we did in the middle east and we recorded the temperature of the heated microscope stage used for looking at the egg and embryos: it fluctuated between 31 and 45 degrees - enough to damage egg spindles from the cold at one extreme and to coagulate egg cytoplasm at the other !

In the future, we can look forward to continuous monitoring of the health of eggs and, especially, embryos, in much the same way as today we monitor newborn babies when they are born.

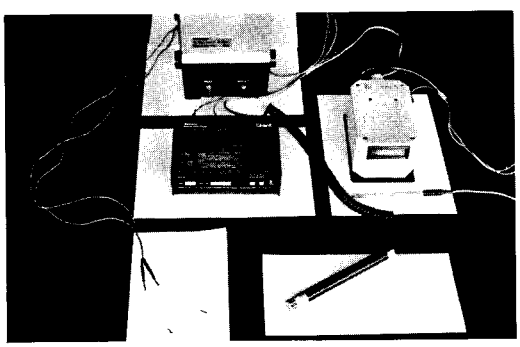

Fig. 5 Sydney IVF microprobes for monitoring temperature (2 channels), pCO_2, pH and relative humidity in the microenvironment of eggs and embryos, and the "squirrel" used for continuous logging of data.

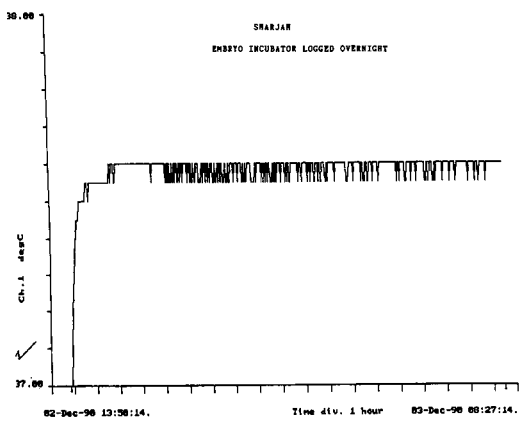

Fig. 6 Temperature monitoring of the embryo section of a water-jacketed incubator. Temperature fluctuations are minimal and acceptable.

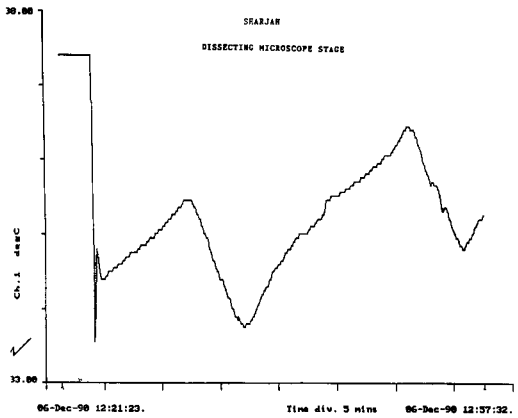

Fig. 7 Temperature monitoring of a heated microscope stage used for identifying oocytes and for preparing embryos for transfer. Damage to oocytes and embryos is inevitable with such fluctuations of temperature.

Cooling of eggs between recovery and entry into the incubator is one of the commonest mistakes we find and it can be very difficult to show where the problem lies (and if it has been corrected) without the solid-state monitoring equipment of the kind developed at Sydney IVF. Without such monitoring the problem may be unsuspected, because the fertilization rates of the eggs affected by cooling may not be reduced; the 2-cell, 4-cell, 8-cell stages may be reached more or less on time; but among the few pregnancies that happen there are many, many miscarriages. The same subtle consequences follow exposure of the early embryo to suboptimal chemical environments.

So clinical IVF programs can believe they are near perfection when they achieve some pregnancies, most of which end in miscarriage. The truth is that very many steps in the process may be suboptimal, but all will require correction before high pregnancy rates follow, and before patients who can pay for IVF stay at home instead of travelling to other countries for their assisted conception.

Acknowledgement

I thank the outgoing Chairman of the Thai College of Obstetricians and Gynaecologists, Professor Vithoon Osathanondh, for the opportunity of speaking to the College, and Organon Thailand (Ltd.) for sponsoring my visit to Bangkok. I also thank Dr. John Anderson, Technical Director at Sydney IVF, for making possible the technical developments described.

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