# RECURRENT MISCARRIAGE IN MALTA:

## An Analysis of 135 Patients Referred to the Miscarriage Clinic

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# CONTENTS

## Section A



## Section B



## Section C

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Recurrent miscarriage affects about 1% oc couples who are trying to have children.
The classical definition of recurrent miscarriage i.e three consecutive losses is an epidemiological one based on risk estimates of a miscarriage following each consecutive loss. This definition does not, however, take into consideration those couples who have already faced two recurrent miscarriages and are desperate to have a child.

When the miscarriage clinic was set-up it was recognized that the management of this condition was subject to the individual interpretation of gynaecologists caring for these patients.
The concept was that all patients should receive the best possible care and with this in mind it was decided that two recurrent miscarriages would qualify for referral and management.

Another concept which the clinic adopted is a comprehensive investigation protocol as opposed to a highly selective one. This ensured that all possible pathologies which may be associated with miscarriage would be identified. The results will show that a number of women were found to have medical problems through this approach.

A particluar attention to modern treatment protocols ensured that patients were prescribed the most effective treatment possible for their condition. The availability of amiscarriage clinic is in itself the most important factor for the largest group i.e. the idiopathic group and guarantees an improvement in outcome which was clearly shown in the results.

In conclusion, the setting-up of a miscarriage clinic improved the care for these patients and contributed to the overall success reported for these unfortunate patients.

# INTRODUCTION

Recurrent Miscarriage is considered to be one of the classical conditions that have been described in Obstetrics and Gynaeclogy.

The standard definition is one where the women suffers three consecutive miscarriages. This definition requires that a proper diagnosis of pregnancy is made in each case and also that the gestational age of the event must be pre-viable. The latter criterion may not be clear in all cases and careful documentation and further study is required as this gestational age is constantly being reduced further. Obviously there must be a lower limit to this reduction. Presently the World Health Organisation definition of a loss below 500gms in weight is the standard to be adopted. This makes comparison of results easier than applying gestational ages.

Miscarriage is a serious situation for the patients to cope with. There are health risks involved and deep psychological problems to face up to, especially when the problem becomes recurrent.

In modern society childbearing is becoming more and more a controlled and planned part of an individual's life.

Unfortunately the tendency is to postpone this part of one's

life until a later time. This state of affairs is making miscarriage a condition of increasing importance to our society.

For a couple faced with a problem of recurrent miscarriage, a solution must be considered a right, if one is available.
The natural instinct towards childbearing have not diminished but the pressures on a woman's life has increased substantially. With these facts in mind every effort must be made in order to determine if a solution exists for that couple, and in a broader context for our society, not to say the species in general.

The group of patients suffering from this clinical problem have very diverse pathological causes and there is also a substantial idiopathic group. This has hindered the study of the condition in terms of aetiology, pathology and clinical features. This difficulty has in turn, made improvements in clinical management difficult to achieve.

# Historical Review

Miscarriage is often a very distressing condition for the parents who often feel so upset that psychological problems as severe as bereavement are experienced.
Reactions of a religious nature are common following a miscarriage and, in The Bible, Hosea - 9 : 14 describes a damnation ' give them a miscarrying womb and dried-up breasts .....'. This illustrates the evil miscarriage was associated with in early cultures.
In contrast God, in the Talmud, is said to protect the orifice of the womb against miscarriage.

The impact of miscarriage is so great that it has been feared since antiquity.
A review of various cultures shows that miscarriage features in all mythologies. After miscarriage, ceremonies of ritual purification for both husband and wife were considered essential in parts of Africa, to prevent a fatal disease developing in either of the parents.
Roman women presented flowers to the temple of Juno in order to avoid a miscarriage while in India prayers were offered to the god-dess Proniparni to protect the conception from the embryo-eating demon Kawa. This demon was known as Kan Kamiak in Borneo and Con Ranh in Indochina (Kuller JA and Katz L 1994).

In biblical times the women of Greece, Mesopotamia, parts of

France and Germany and ancient Israel wore an ' eagle stone' to prevent miscarriage; this rattled when shaken and was worn on an armlet.

Soranus of Ephesus (98- 138 AD), who practiced in Rome, wrote his famous work Gynaecology . He realized the importance of age to childbearing (a most capable mother was between 15 and 40 years old), presumably also in terms of increasing miscarriage rates with increasing age.
He also described factors which may cause a miscarriage : 'forced detention of breath, coughing, sneezing, blow and falls.....lifting heavy weights, leaping, sitting on hard sedan chairs...want....drunk-enness, ...flow of blood from the nose (I.XIV.46). A miscarriage in the first month of pregnancy was called an 'abortion of the embryo' by Soranus, and was indicated by the explusion of ' a clot of blood, or some piece of flesh, unformed or formed depending on the stage (I.XVIII.59).

Care of the woman during this seed-preserving stage included con-finement to bed for two days, anointment with freshly ground oil from unripe olives, and a light diet of grains and cereals. She was to abstain from taking baths or drinking wine for seven days, since the relaxation of the body was thought to enfeeble the structure of the seed. Exercise was gradually increased, as well as food, but she was to eat neutral foods that were not greasy or acidic.
Furthermore, she was to avoid intercourse, because it was thought to

disturb 'thc various parts about thc uterus which need rest' (I.XIV.46)

The Hippocratic school on the other hand ordered great deal of garlic or the pistils of the 'silphium' plant to prevent miscarriage, for the sap of these plants was supposed to produce wind and everything that causes flatulence is, in their opinion, beneficial for pregnancy.

Culpeper in his directory for Midwives (1651) believed that women were most likely to suffer miscarriage in the first two months of their conception and that many were delivered at the end of seven months because of the completeness of the time, seven being of a Note of perfection'. A favourite treatment was syrup of tansie, "a most excellent medicine, though it is not in the Colledges Worm-eaten Dispensary for the herb by the magnerick verrue draws the Child in the womb anyway, or retains it in its proper place." He also refers to the previously mentioned nostrum the AEtitres or eagle-stone (from O'Dowd 2001).

In the middle ages, diet and nutritional factors were considered to be a major cause of pregnancy loss.

Miscarriages have also played an important role in history: in England in the 16$^{th}$ and 17$^{th}$ century the recurrent miscarriages of Anne Boleyn and Catherine of Aragon had a prominent impact on the succession of the monarchy.

From the Textbook of Midwifery by Professor Otto Spielgelbereg (translated from the second German Edition by JB Hurry and pub-

lished in London by The New Sydenham Society 1887) a distinction between miscarriage and premature labour is clearly made based on the viability of the fetus judged to be at 28 weeks. Miscarriage was already recognized to be much commoner than reported and it reports '*it is well known that women who have aborted, are apt to abort again in subsequent pregnancies, and at about the same period as before (habitual abortion). This impotentia gestandi may be due to recurrent fetal death, retroflexion, uterine tumour, disease of the mucous membrane or disease of the generative system. One must also admit an undefined constitutional irritability of the uterus, which only allows it to reach to a certain degree of development* (possibly a bicornuate uterus).

When considering causes of miscarriage Spielgelberg describes those cases where no cause can be found and proposes a special irritability of the uterus or an undemonstrated inherited predisposition. He argues against too young or too old an age as being causes of miscarriage; in the latter he was to be proven incorrect. *Prophylaxis* against miscarriage required that the most accurate information of the factors concerned in the aetiology and apply effectual treatment. When this was not possible prolonged abstinence from intercourse was recommended. The woman was advised to keep as quite as she could without interfering with good digestion special care being taken on those days when the previous miscarriage took place.

One of the earliest reviews of the specific problem of miscarriage is by Jacob Heinrich Borrel ; de abortus aetiologica - dissertatio inauguralis medico - obstetrica 8vo Berlin ; typis natorffiaris 1837. It was recognised very early on that fetuses from abortion material very often revealed abnormalities. This was probably the first correct observation regarding one of the causes of miscarriage.

Weinzierl in 1933 attempted a definition of habitual abortion, defining it as not only a series of successive miscarriages but also one in which the aetiology is unknown. In the same year Wagner suggested that habitual abortion was due to hypofunction of the ovaries, in particular, of the corpus luteum. This could at the time be administered both orally and intra-muscularly. This was also the time when it was recommended that a diet to help prevent recurrent miscarriage should include iodine, calcium, vitamins and arsenic (the practical Medicine Year Book Obs\Gynae 1933 - J. B. Delee and J. P. Greenhill eds). Thyroid replacement was also proposed as a cure at this time.

Following on the report regarding the positive effect of the corpus luteum extract administration, Hall G.J. reported the successful treatment of a case of habitual abortion with progestin. This is a therapeutic option which was to persist to the present day. The contemporary suggestion that vitamin E (as wheat-germ oil) and vitamin C had a favourable effect on the outcome of habitual abortion was however to fail the test of time.

Conditions such as syphilis which were important in the past in the aetiology of the condition are rarely encountered nowadays.

By 1950 congenital or acquired abnormalities of the uterus (sub-mucous myoma) became a recognised cause of recurrent abortion and a hysterosalpingogram was recommended in all cases ( Sanchez Ibanez - rev espan obst. Y ginec. 8:451, 1949). Endocrine causes were also considered one of the foremost causes, but investigation was difficult until the development of radio-immuno assays and was by a pre-menstrual curettage rather than a serum assay of the reproductive hormones.

In Malta, one of the earliest documents on miscarriage is to be found in the periodical 'Il Barth'. This was a journal published by Dr Gavino Gulia, and issued every fourty days. On the edition of the $10^{th}$ of June 1872, reference is made to Dott Gaetano Laferla who since 1858 had encouraged his colleagues to report cases of miscarriage and premature delivery to him in an effort to study the problem in Malta. It is interesting to note that this population of patients was made up of 17 who miscarried in the first two months of pregnancy and 63 between the second and fourth month of pregnanacy (there were 18 premature deliveries). In the absence of exact knowledge of how many miscarried in the third month, it would seem that the distribution of the gestational age at miscarriage has changed.

# THE INTRODUCTION OF A MISCARRIAGE CLINIC

The concept of a dedicated clinic is the result of the development of sub-specialisation in obstetrics and gynaecology as in other spheres of Medicine.

With the great advances in knowledge in all aspects of medicine it has been considered inopportune to persist with the classically acknowledged specialist fields of competence.

Following the development of sub-specialsiation, the development of dedicated clinics having their own particular set-up, as best suited to the problem being addressed became an important aspect of expansion of gynaecology services.

A dedicated miscarriage clinic is possibly the best application of the concept of a sub-speciality clinic. This is because of the very intensive investigation and counselling sessions that are required with this condition. It will be re-iterated a number of times that recurrent miscarriage is a condition where it is very often difficult to make a specific diagnosis and therefore offering treatment is a delicate and often difficult matter.

# The Clinic

The Miscarriage Clinic was one of the first sub-speciality clinics to be set-up in the Department of Obstetrics and Gynaecology.

The policy was that introduced by Professor M.P. Brincat on whose initiative I was able to set-up the service.

The clinic was, and is, sited in the out-patients' section of the Obstetrics & Gynaecology department and is held on a weekly basis.

Patients are referred by their respective Consultants and following investigation, counselling and advice are referred back to their Consultant with a plan of action in case of a subsequent pregnancy.

It is the experience of many that most patients with recurrent miscarriage will come to depend 'heavily' on the staff of the miscarriage clinic and if at all possible their needs are seen to by these same doctors.

It is now time to expand on the services of the miscarriage clinic and I hope to make the case for this expansion in this dissertation and to detail them in the recommendations at the end.

After an initial period of introduction the clinic gained in popularity both with the patients and with medical and paramedical staff.

This was a new concept of referral within a department, from

Consultant to Consultant, which obviously did require some convincing and very proper methods being adhered to.

Once the advantages of this method of dealing with 'special situations' became clear to all, work increased considerably, especially since there was a backlog of patients who needed to be seen to.

# AIMS

The M Phil thesis is a descriptive study of the patients who were followed-up at the miscarriage clinic over the period 1996 to 2001.

This is the first miscarriage clinic to be set-up in Malta and through this study group the nature and extent of the problem in our gynaecological population, together with a thorough analysis of the management of multi-aetiological condition, will be described and discussed.

# MATERIALS AND METHODS

The subjects of this study were couples referred to the Miscarriage Clinic.
Patients are referred by their respective Consultants for assessment following a history of Recurrent Miscarriage.

Although the classical definition of Recurrent, Habitual, Miscarriage is three consecutive miscarriages this was considered too strict and patients were accepted following a history of two recurrent miscarriages.

## 1. THE FIRST INTERVIEW

At the first interview couples were assessed as to their eligibility to be seen and managed by the miscarriage clinic.

### CRITERIA

Patients should have suffered at least two recurrent miscarriages. They may have had a child before the miscarriages but not in between.
Ideally couples should also wish to conceive and have children in order to justify the expense.

The interview was run by going through the history using a standard interview sheet. Details about the past miscarriages

were recorded in detail.

General information about the patients' history were examined and recorded.

At the end of the first interview a clear picture of the couples miscarriage problems was usually apparent. This served to counsel the couples as to what was to be expected, both from investigation and possibilities for treatment.

For the purposes of the clinic the causes of recurrent miscarriage had been listed as :

Anatomical
Endocrinological
Genetic
Immunological
Infectious
Thrombophilia
Unexplained

Investigation for thrombophilia was only introduced in the last year of the study so data on this will not feature in the analysis section. It is now a standard investigation for recurrent miscarriage though its position in recurrent miscarriage is still under study.

# 2. THE INVESTIGATIONS

## ENDOCRINOLOGICAL

An endocrinological diagnosis is made through a hormone profile. Endocrinological causes are further sub-divided into polycystic ovary syndrome and luteal phase defect .

Luteal phase defect is diagnosed when the luteal phase progesterone is low, below 30 nmol/l. This is repeated on at least one occasion in order to make the diagnosis.
Hormone assays are chemi-luminescent/immunoassays.

Polycystic ovarian disease is diagnosed by a combination of hormone assay and ultrasound examination.
Hormone assay should feature a raised LH to FSH ratio, a raised testosterone level and usually anovulatory cycles. The oestradiol level is usually normal. Ultrasound examination should present the classical appearance of polycystic ovaries, i.e. numerous micro-cysts in the sub-cortical area.
Ultrasound was usually by a trans-abdominal 3.5MHz probe though on occasion a trans-vaginal 5 MHz probe was used, especially when obese.

## ANATOMICAL

Anatomical anomalies are divided into mullerian fusion anomalies, cervical incompetence and fibroids.

The reasons for this sub-division is that the management of the various conditions is somewhat different.

A diagnosis of a mullerian fusion anomaly can be made when a hystero-salpingogram is performed or rarely when the uterine cavity is opened at surgery eg at caesarean section or hysterotomy.

Expert ultrasonography with high resolution equipment can diagnose a septate uterus but the 'gold standard' investigation must be the hysterosalpingogram at this point in time.

Since the latter is invasive and associated with significant patient discomfort apart from the risk of pelvic sepsis a sensible approach would be to screen with ultrasonography and perform a hystero-salpingogram when this is suggestive of an abnormality.

Cervical incompetence was diagnosed by one of three methods or combination of evidence from each. These were a suggestive clinical history, hystero-salpingography and the state of the non-pregnant cervix (if it allows a hegar 8 dilator to pass with ease it is deemed to be incompetent).

## AUTOIMMUNE

Autoimmune causes of recurrent miscarriage are characteristically those patients who are positive for antiphospholipid antibodies. These are anticardiolipin antibodies and lupus-anticoagulant.

The anticardiolipin antibody test is an ELISA assay for immunoglobulins G and M. The test result must be interpreted in relation to the normal value and are often described as being moderately or highly significant.
The test should be repeated after a period of six weeks in order to confirm the diagnosis.

***The laboratory testing for Lupus Anticoagulant has proved to be more difficult. The minimal criteria for the detection of LA were proposed by Triplett & Brandt in 1989:***

1. A prolongation of a phospholipid dependent screening test such as the APTT
2. Demonstration that the abnormality is due to an inhibitor (rather than a factor deficiency)
3. Proof that the inhibitor is directed against phospholipids.

Screening tests have been used for lupus anticoagulant and three have been employed. The most commonly applied is the dilute APTT (activated partial thromboplastin time) when an

activator and a phospholipid mixture (partial thromboplastin) trigger the contact phase of coagulation in the plasma sample. LA will bind to the phospholipids and prolong the assay. When prolongation of the APTT is due to a coagulation factor deficiency, the clotting time corrects when the test is repeated on an equal mixture of patient and normal plasma, whereas the prolongation will persist in the presence of LA. A weak antibody may be diluted out and its presence undetected. Inhibitors to clotting factors, usually factor VIII, are associated with bleeding rather than thrombosis but also cause prolongation of the clotting times which may not be corrected by the addition of normal plasma. There is great variability in the composition of APTT reagents. The characteristics of the phospholipid component appear to be critical in determining LA sensitivity and reagents vary both in the type of phospholipid present and their relative concentrations (2). This results in inconsistent sensitivity (3). Also acute phase reaction and pregnancy are associated with increased levels of fibrinogen and factor VIII, which tend to shorten the APTT and could mask a weak lupus anticoagulant. It is essential that sensitive reagents are employed and even then a normal APTT may be insufficient to exclude LA.

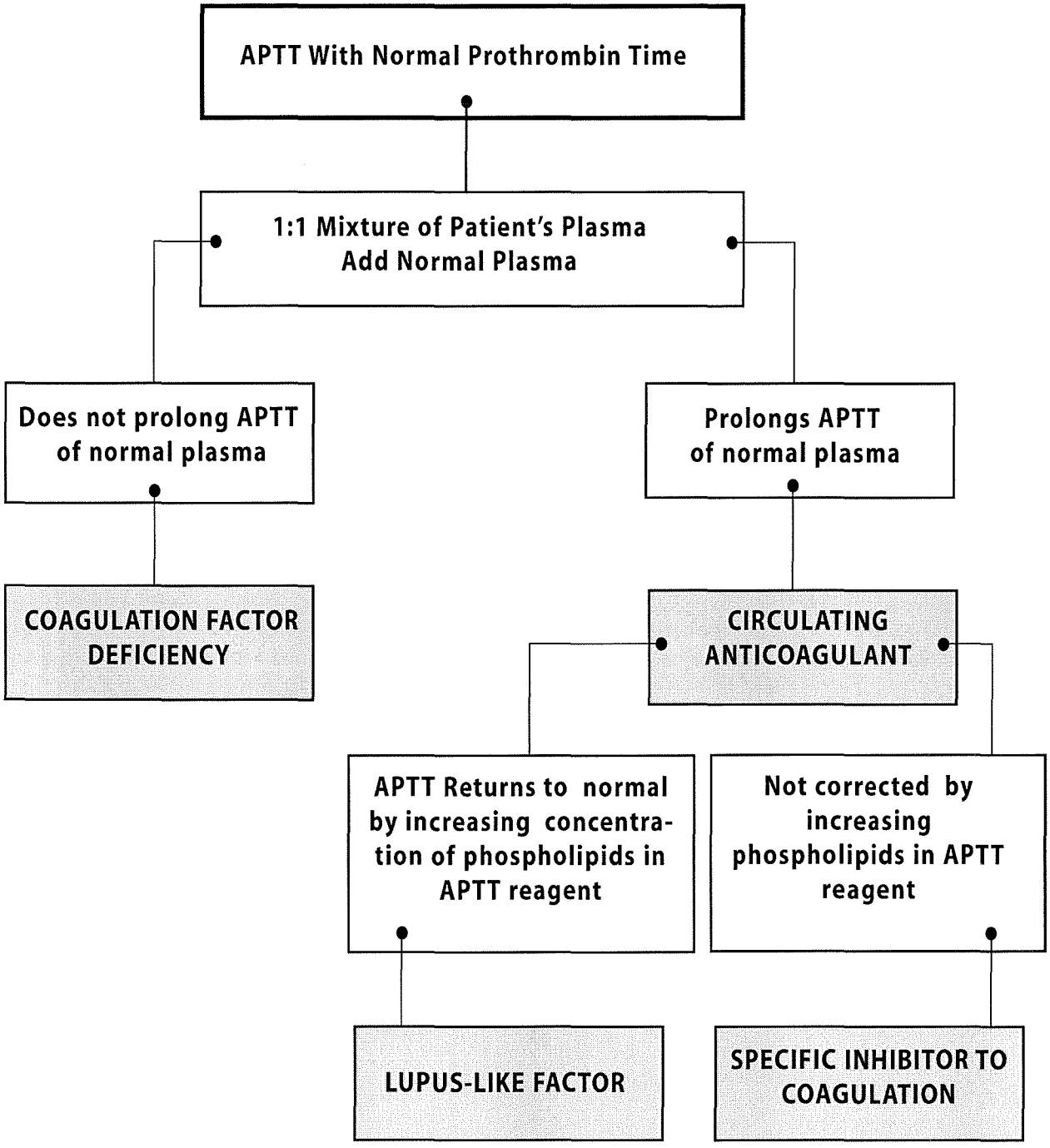


**Fig. I** Flow diagram describing the evaluation of patients with abnormal APTT, circulating anticoagulants or factor deficiencies (after Reece EA 1984).

The flow diagram depicts a single method of evaluating patients with abnormal APTT: circulating anticoagulants or factor deficiencies can be investigated by this means (4).

**The kaolin clotting time (KCT)** is another test where the contact phase of coagulation is triggered by kaolin and no exogenous phospholipid is added. The sensitivity to LA is theoretically enhanced because the small amount of phospholipid present is only that derived from residual platelets in the test sample and plasma lipids. The test is also affected by clotting factor deficiencies and anticoagulants, but LA is identified when the KCT fails to correct even after relatively large proportions of normal plasma are added, whereas in factor deficiency the KCT is corrected with small amounts of normal plasma. Usually, control and patient plasmas are tested, as well as one mixture (8o% control ; 20% test). A test/control ratio >1.2 generally indicates an abnormal result, and a mixture ratio of >1.2 should be considered as indicative of LA.

In the **DRVVT,** Russell's viper venom activates factor X, which in turn activates prothrombin in the presence of calcium ions, factor V and phospholipid, leading to the formation of a fibrin clot. This test does not involve the clotting factors of the intrinsic system, unlike the APTT, nor factor II, like the prothrombin time. Any inhibition of the coagulant active phospholipid by LA results in a prolonged DRVTT. Like all LA tests it is not specific but is as sensitive as KCT (5). The specificity is improved by repeating the test in the presence of

a high concentration of phospholipid. This should result in partial or complete correction of the prolonged clotting time due to LA.

In order to confirm the presence of an inhibitor the test is repeated using a mixture of the patient's plasma with normal plasma. If the test is due to an inhibitor the test will remain prolonged. If the abnormality is due to a factor deficiency the normal plasma will act as a source of the factor and the test will correct to normal. Lastly a platelet neutralization procedure (PNP) is used to confirm the antiphospholipid nature of the inhibitor. Lysed platelets are added to the abnormal plasma, and the screening test (APTT or dRVVT) is repeated. An abnormality caused by LA will correct in a PNP whilst an abnormality due to a factor will not.

As with all coagulation tests, because of variations in reagents and techniques, it is essential that laboratories derive local normal ranges using a large number of plasma samples from healthy volunteers.

The following points will have to be considered when interpreting tests for antiphospholipid antibodies :

1. a firm diagnosis of APS requires a clear clinical history and persistently positive test results for aPL
2. LA tests are indirect, not entirely specific and of variable sensitivity
3. more than one type of LA test will need to be done to make the diagnosis accurately

4. anticoagulant therapy will prolong clotting times and interfere with the detection of LA
5. anticardiolipin antiboby tests are sensitive but transient positive test results are common
6. both LA tests and aCL (IgG and IgM) assays are required to diagnose or exclude APS

In order to avoid misinterpretation of test results requires knowledge of their accuracy, reproducibility, sensitivity and specificity.

During the period of study the testing for lupus anticoagulant has evolved from a simple dilute APTT to direct testing of the specific antiphospholipid in recent times. This is now accurate and reliable.

## KARYOTYPING

Parental Karyotyping was indicated by the investigation protocol adopted in patients who suffered at least three recurrent miscarriages.

Patients who tested positive were referred for genetic counselling which was arranged by consultation with the Genetics Clinic under Professor Alfred Cuschieri.

# RECURRENT MISCARRIAGE CLINIC

**NAME:** **Age:**
**HUSBAND/PARTNER:** **I.D. no.:**
**ADDRESS:** **Tel. no.:**
**REFERRING DOCTOR:** **Tel. no.:**

**PAST MEDICAL HISTORY:**
MEDICATION: DRUG/ALLERGIES:
FAMILY HISTORY: Miscarriage/Infertility:
OCCUPATION: Smoker/alcohol

**GYNAECOLOGICAL HISTORY:**
Menarche: cycle: duration/loss
LMP:
Oral contraceptives: Last used:
Fertility drugs:
**DO YOU CONSIDER YOURSELF FERTILE?**

**MISCARRIAGE DETAILS**

| DATE | GESTATION | SYMPTOMS | D&C | COMMENTS |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

OTHER COMMENTS:

**BP__ / __**

**PA**

**PV**

**CLINICAL EXAMINATION:**

**Fig. 2** Copy of the form used to take the history and patients details at the first interview.

## 3. THE TREATMENT PROTOCOLS

The treatment protocols employed were the following :

1. low-dose aspirin only (75 mgs)
2. aspirin and prednisolone (15 mgs)
3. aspirin and heparin (5000 units bd)
4. aspirin and duphaston ( 10 mgs tds)
5. duphaston and human chorionic gonadotrophin (5000 units twice weekly)
6. uterine surgery
8. cervical cerclage

Luteal Phase Defect: Clomiphene citrate 50mgs day 2-6 followed by dydrogesterone 10 mgs tds from conception.

Polycystic Ovary Syndrome: clomiphene citrate 50 mgs daily day 2 to 6. Increasing dose of clomiphene to 150mgs if necessary and luteal phase support with dydrogesterone 10mgs tds.

In severe cases, resistant to clomiphene, human menopausal gonadotrophin was used with prior down-regulation with GnRH analogues.

Congenital uterine abnormalities were treated with dydrogesterone till 20 weeks, followed by a combination of ritodrine

2.5 mgs 4 hourly and nifedipine 10mgs bid.

A cervical cerclage was also employed.

This was the same protocol used for cases with cervical incompetence.

This was a protocol developed for the management of high order multiple pregnancy but found equally satisfactory application for congenital abnormalities of the uterus.

The initial protocol for antiphospholipid antibody syndrome was either aspirin alone or a combination of aspirin and prednisolone.

This was subsequently altered to aspirin (low-dose 75mg daily) and heparin (unfractionated 5000 units sc bd).

All patients were prescribed folic acid 400 micrograms daily.

## 4. POSSIBLE OUTCOMES

1.No further conceptions

2.Another miscarriage on no treatment

3.Live baby on no treatment

4.Another miscarriage on treatment

5.Live baby on treatment

Results were recorded according to the above criteria.

**Key to abbreviations**

NAD : no abnormality detected

NO R : no treatment prescribed

LDA : low dose aspirin

PRED : prednisolone

ACA+ : anticardiolipin antibody positive

APS : antiphospholipid antibody syndrome

HEP : heparin

PCOS : polycystic ovary syndrome

HMG : human menopausal gonadotrophin

HCG : human chorionic gonadotrophin

C&T : cercalge and tocolysis

TOCO : tocolysis

# RESULTS : INDEX

## PATIENT CHARACTERISTICS

Age
Past History
Treatment History
Infertility
Family History
Alcohol use
Smoking
Social & Occupational
Age at Menarche
Menstrual Cycle

## HORMONAL TREATMENT

Oral Contraceptive Use
Clomiphene citrate
Human Menopausal Gonadotrophin

## MISCARRIAGE HISTORY

## RESULTS OF INVESTIGATIONS

## OUTCOME OF TREATMENT PROTOCOLS

## STATISTICAL ANALYSIS OF RESULTS

# RESULTS

**The total number of patients who were eligible for analysis, bearing in mind their clinical history and the completeness of the data accumulated about them was 135.**

## SOCIAL and PERSONAL DATA

# RESULTS

The total number of patients who were eligible for analysis, bearing their clinical history and the completeness of the data, accumulated about them was 135.

## SOCIAL CHARACTERISTICS

### Marital Status.

The very nature of the medical problem i.e. recurrent miscarriage implies that the patient is seeking to have a child.

In fact, all but two of the patients studied were married. The remaining two were in a stable relationship and in no case was there a change of partner throughout the period of study.

## Occupation

The following is a list of occupations which the patients held at the time of referral.

Table 1. The Occupations of the patients of the study group

| OCCUPATION | NUMBER OF PATIENTS |
|---|---|
| Housewives | 83 |
| Machine Operators | 12 |
| Clerks | 9 |
| Teachers | 6 |
| Secretaries | 4 |
| Cleaners | 4 |
| Bank employees | 3 |
| Waitresses | 2 |
| Telephone operators | 2 |
| Sales representatives | 2 |
| Hairdresser | 1 |
| Auditor | 1 |
| Supervisor | 1 |
| Nurse | 1 |
| Supermarket assistant | 1 |
| Chambermaid | 1 |
| Chef | 1 |
| Postal worker | 1 |
| Total | 135 |

# AGE

The age range of the group of patients under study was 19 to 44 years with a mean of 31.6 years. This value is slightly higher than the mean age for mothers in 2002 (27.9 - NOIS).

This range of the ages of the patients would reflect the normal reproductive years. The mean age, however, confirms the widely held axiom that after age 30 reproductive failure is more common. Some authors have referred to age 30 as a watershed in reproductive life. That the mean age of mothers suffering a miscarriage is above 30 and higher than that of mothers having full term pregnancies confirms the importance of age in this problem.

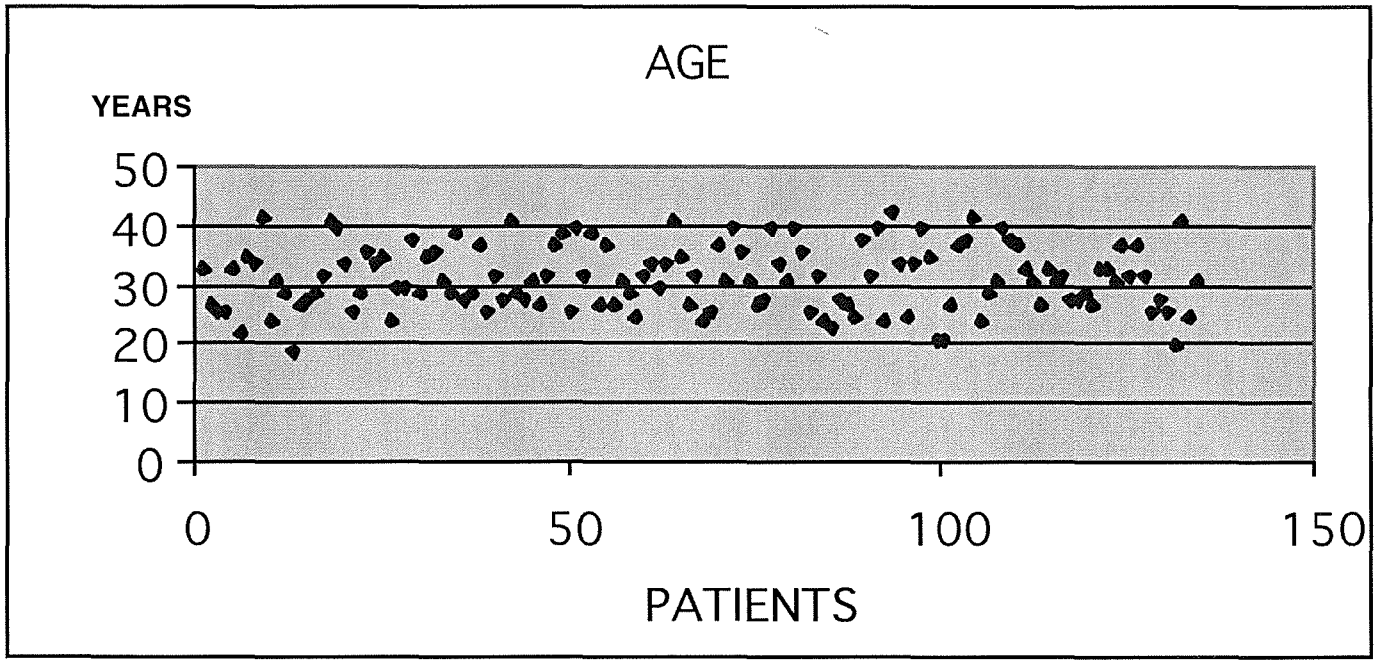


**Fig. 3** The distribution of the age at referral of 135 patients referred to the miscariage clinic

Many factors can contribute to this fact but biologically age-related reproductive failure can best be explained as being due to problems with the resumption of meiosis which occurs after ovulation and leads to chromosomal aberrations such as trisomies due to non-dysjunction. This is also reflected in a poorer outcome with increasing age.

Delaying childbearing is not only an infertility issue but also one of increasing miscarriage rates.

# THE MENSTRUAL CYCLE

The mean length of the menstrual cycle in the group of patients under study was 30.3 days. This is only slightly longer than average, the mean length being generally quoted as 28 days.

Since this group of patients represents a heterogenous group in that the causes of recurrent miscarriage varied between individuals, the group of patients with oligomenorrhoea represent those suffering from polycystic ovary syndrome. This was not a large group with 16 cases representing 11.85% of the group.

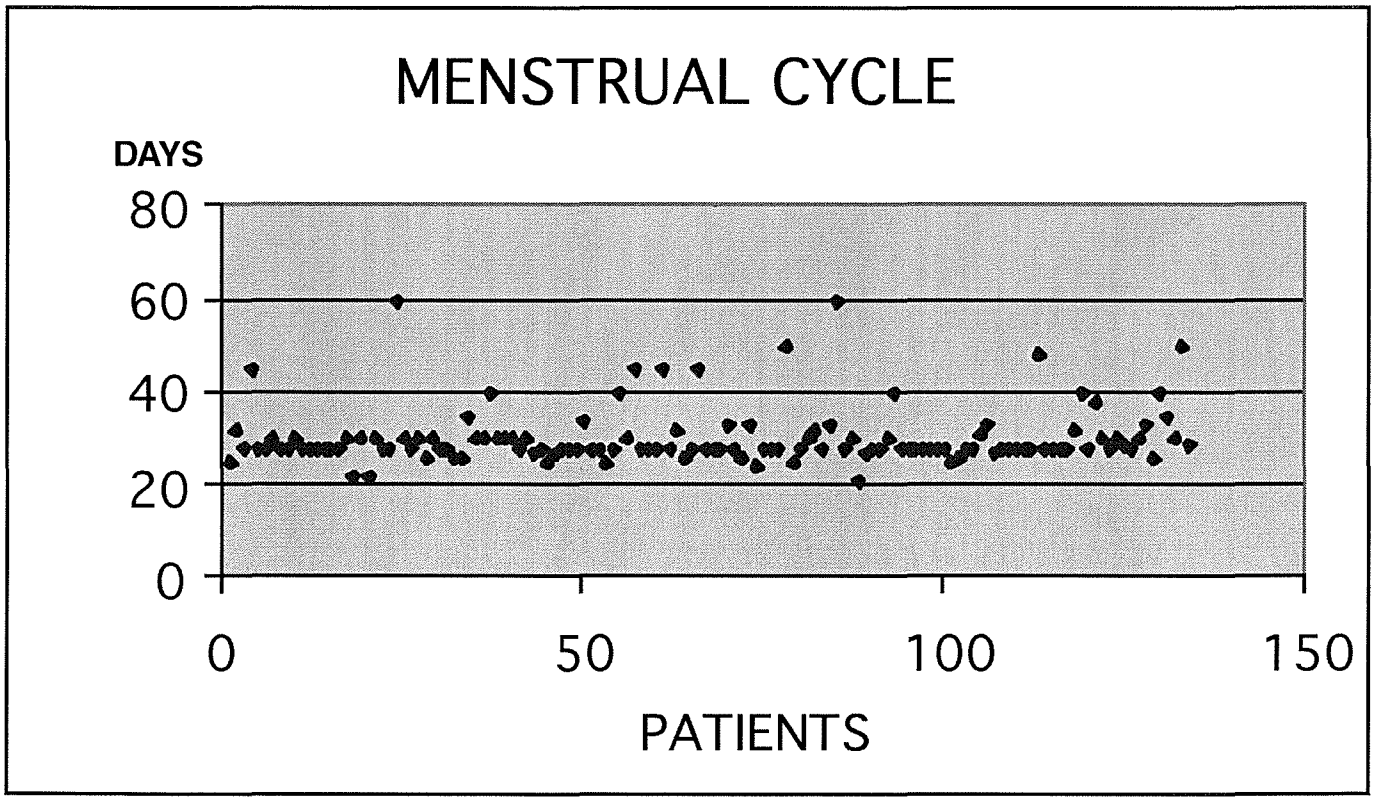


**Fig. 4** The distribution of the menstrual cycle in 135 patients referred to the miscarriage clinic

This, however should account for the slight increase noted.

The graph describes a fairly even distribution around 28 days with scatter mainly above this, and in only in a few cases less than this.

The main clinical application of the cycle length is that oligomenorrhoea has been reported to be associated with improved full term pregnancy rates when human chorionic gonadotrophin is used in the management (71).

# AGE AT MENARCHE

The mean age of the menarche was 12.4 years. This is again close to what would be expected in the general population. Delayed puberty can be a feature of polycystic ovary syndrome and the cluster of patients who had their puberty at around age fifteen may represent this group of patients, also referred to in the section describing cycle length.

The menarche is an endocrinological event and a significant section of recurrent miscarriage is related to endocrinological conditions.

It is in this group that this characteristic (menarche) may be relevant as a risk factor for recurrent miscarriage.

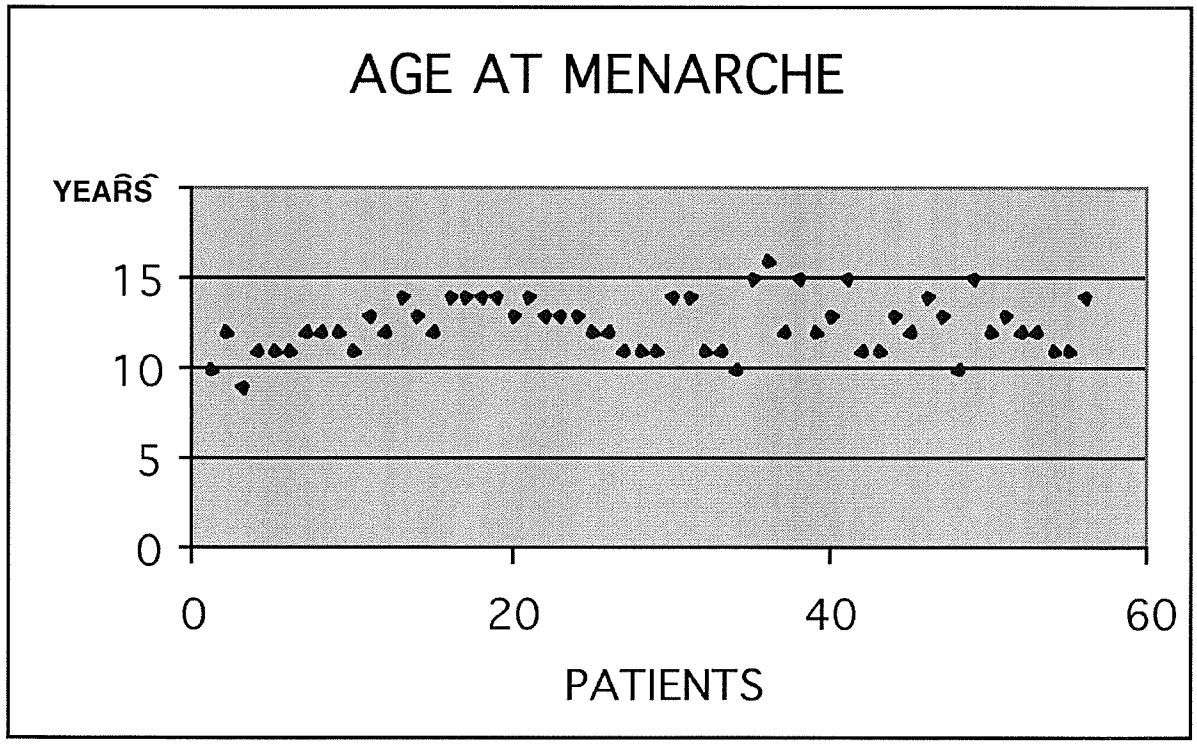


**Fig. 5** The distribution of the menarcheal age in 135 patients referred to the miscarriage clinic

# SMOKING HABITS

| Smoking Habits | Miscarriage Group | Mothers (2002) |
|---|---|---|
| NIL/day | 88 | 3691 |
| <5/day | 14 | 23 |
| 5 – 10/day | 14 | 100 |
| 10 – 20/day | 7 | - |
| > 20/day | 22 | - |
| **TOTAL** | 135 | 3833 |

**Table 2.** A comparison of smoking patterns between normal mothers for 2002 and the study group.

42.2% of patients in the miscarriage group were smokers as opposed to only 3.3% (approximately, because of incomplete data) of mothers having babies for 2002 (NOIS).

This is an extremely significant level of difference but possibly there may be an explanation for this.
Patients who have suffered a miscarriage will be keen to have an explanation for the miscarriage and are likely to report accurately any possible deleterious factor, if not over-report. Normal mothers are on the other hand likely to under-report these factors.

That this may reach this level of difference without there being a direct effect of smoking on miscarriage rates is how-

ever, suspicious.

The increased rates of smoking may also reflect a tendency to anxiety which recurrent miscarriage is known to provoke.

# ALCOHOL CONSUMPTION

45.9% of patients in the miscarriage group admitted to varying degrees of alcohol consumption.

Surprisingly only 2 mothers were recorded as consuming any level of alcohol for 2002 (NOIS).

| Alcohol Consumption | Miscarriage Group | Mothers (2002) |
|---|---|---|
| Nil | 73 | 3816 |
| Occasional consumers | 34 | - |
| Daily 1-2 units | 25 | 2 |
| Daily >2 units | 3 | - |
| **TOTAL** | 22 | 3873 |

**Table 3.** A comparison of alcohol consuption between normal mothers for 2002 and the study group.

It is very likely that this phenomenon is due to motivation on the part of the patient who has suffered a miscarriage to report everything and anything which may give her an explanation for this totally unexpected and negative event.

# RESULTS

## The Relationship between Gestational Age at Miscarriage and the Miscarriage History.

# GESTATIONAL AGE AND ORDER OF MISCARRIAGE

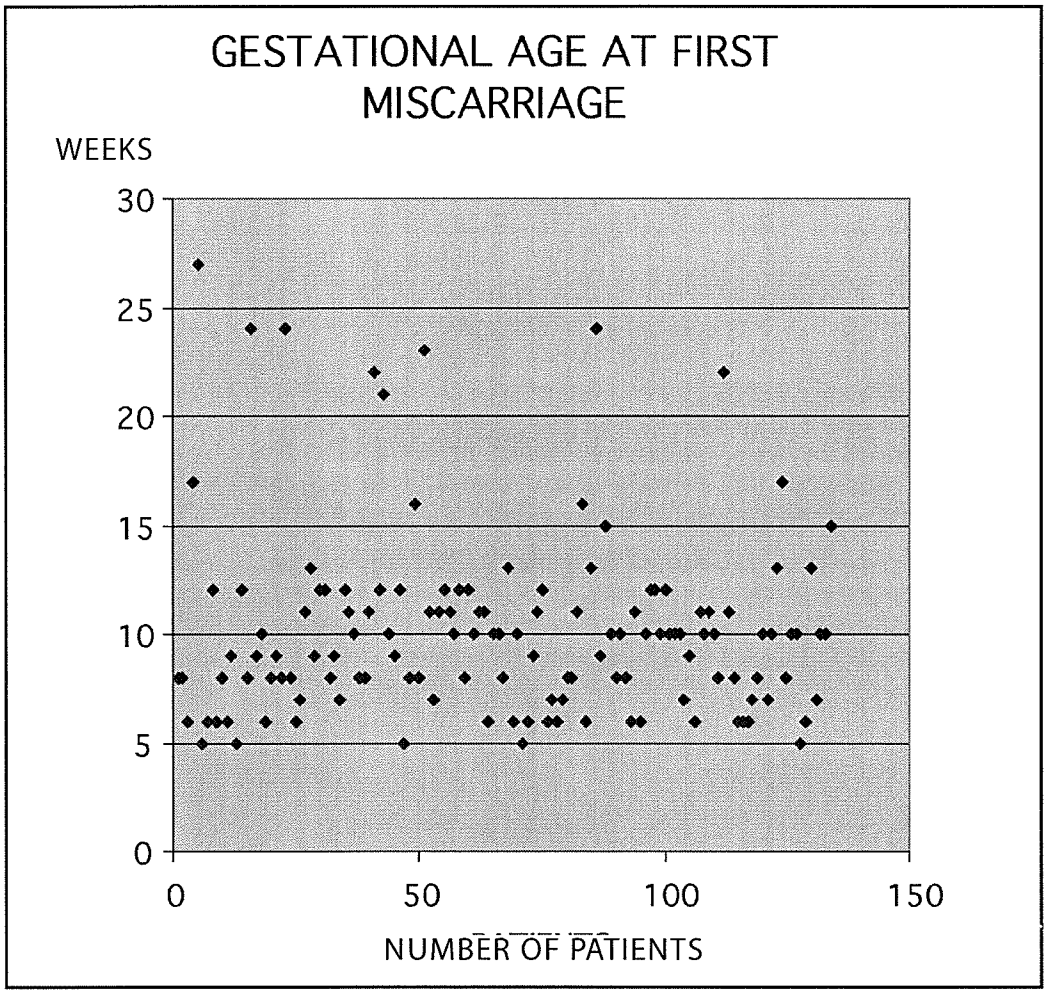


**Fig. 6** The distribution of the gestational age at first miscarriage in 135 patients referred to the miscarriage clinic

The relationship of the gestational age at which subsequent miscarriages occurred in this group of patients suffering from recurrent miscarriage is described below.

The order is taken up to the fourth miscarriage as only a few miscarriages occurred beyond that.

The first miscarriage occurred at about 12 weeks gestation in

the vast majority of cases.

Only eight losses occurred after 20 weeks gestation and six later than 16 weeks i.e. 10.4%.

Again, in the case of a **second miscarriage,** by far the majority of cases occurred in the first trimester

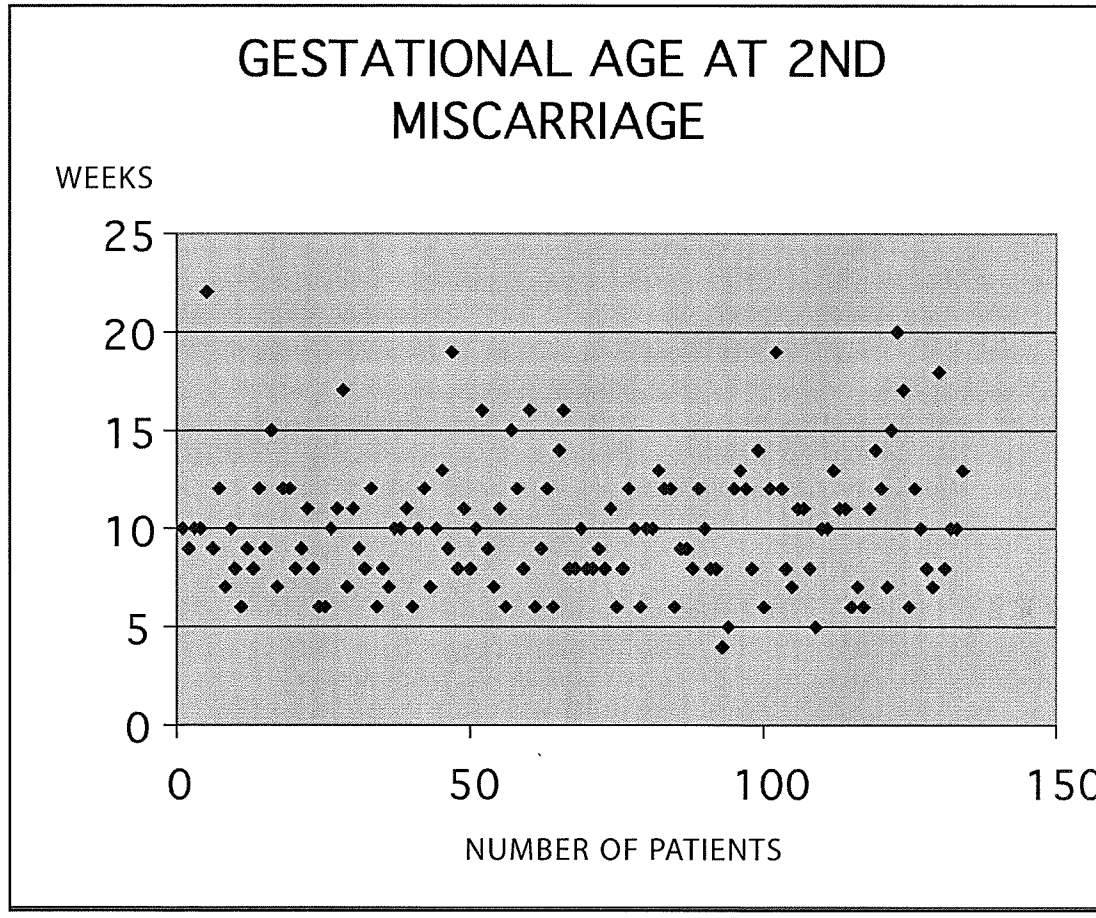


**Fig. 7** The distribution of the gestational age at second miscarriage in 135 patients referred to the miscarriage clinic

There was, however a slight increase in the number of cases occurring at a later date; 12.6%.

There was furthermore an increase in the number of cases occurring later than 10 weeks gestation.

The relevance of this is not clear but 10 weeks represents the end of the embryonic period by which time most causes of spontaneous miscarriage would have resulted in fetal demise. This is likely to be the case in a number of patients with unexplained recurrent miscarriage.

**Pathologies resulting in TRUE recurrent miscarriage seem to cause miscarriage later than those in spontaneous miscarriage.**

There were 60 cases who suffered at least **three consecutive miscarriages.**

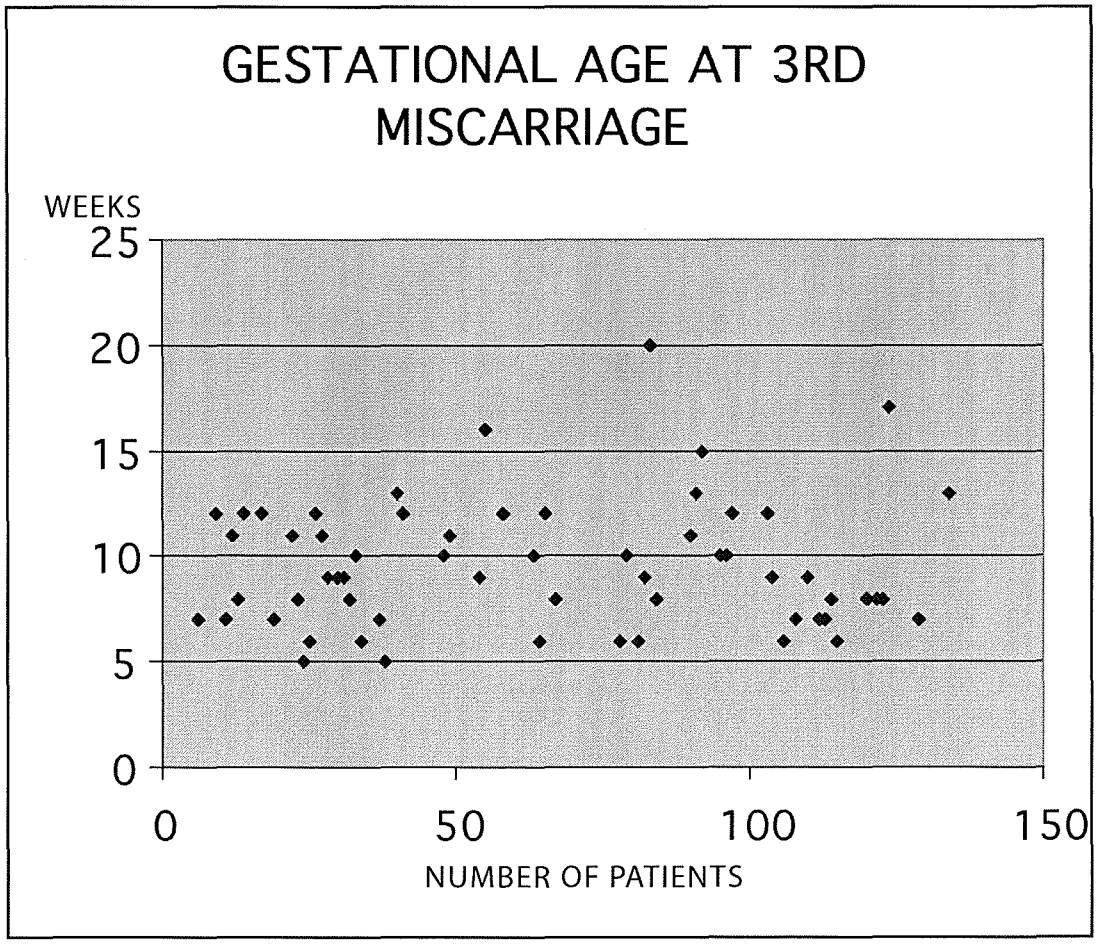


**Fig. 8** The distribution of the gestational age at third miscarriage in 135 patients referred to the miscarriage clinic

Only four of these occurred between 15 and 20 weeks gestation, while the vast majority again occurred in the first trimester.

A potential shift in the gestational age with higher order of miscarriage was not noted in this group and undoubtedly a few of these miscarriages will again be due to non-recurrent causes in spite of being the third in a row.

Yet again the vast majority of cases occurred during the first trimester of pregnancy.

There were 12 cases who had suffered **four recurrent miscarriages.**

These were practically evenly distributed around ten weeks gestation but only one was higher than 15 weeks.

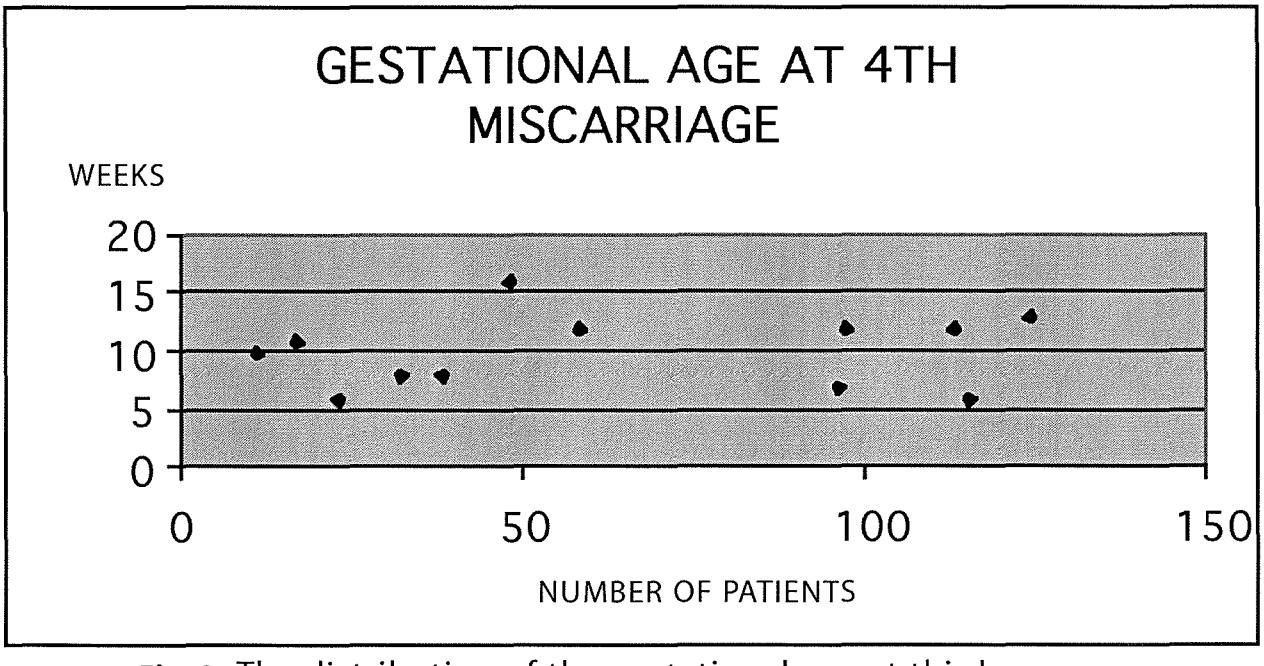


**Fig. 9** The distribution of the gestational age at third miscarriage in 135 patients referred to the miscarriage clinic

Most late miscarriages occur due to anatomical anomalies, either cervical incompetence or bicornuate uterus. After three miscarriages most of these would have been identified and hopefully managed appropriately.
These would not be represented in this graph. Also cases of anti-phospholipid antibody syndrome, also a cause of late miscarriage, will probably be eliminated and it is mostly in the group with two miscarriages that these will be represented as it is then that the diagnosis is likely to be made.

Keeping this in mind the proposal that a higher gestational age at miscarriage is a risk factor for recurrent miscarriage as opposed to spontaneous probably holds true.

# RESULTS

## The Endocrine profile of the patients studied

# Serum Follicle Stimulating Hormone (FSH)

The serum FSH levels were well within the normal range indicating normal ovarian activity.

A single raised FSH level recorded was not confirmed on further testing.

No cases of premature ovarian failure were diagnosed.

The mean FSH level was 6.12 units \L.

Serum FSH levels will reflect ovarian E2 secretion. The level may be used in relation to the LH level in the diagnosis of PCOS.

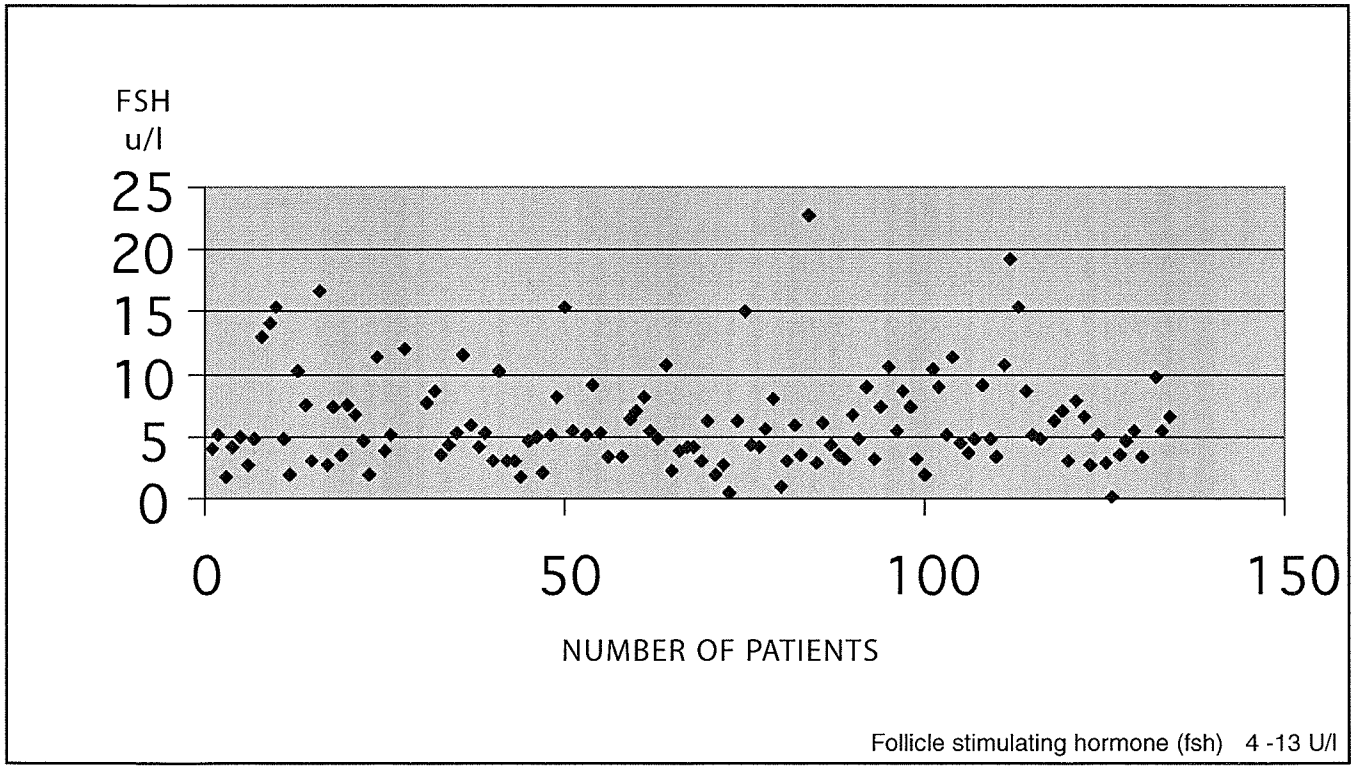


**Fig. 10** The distribution of FSH levels in 135 patients referred to the miscarriage clinic

# LUTEINISING HORMONE

The distribution of luteinising hormone was in the main within normal limits.

A single value was well out of the range (high) and this was probably an ovulatory level in a patient with oligomenorrhoea.

The five plots which were higher than average represented patients with PCOS where an elevated LH level is to be expected and also considered to contribute to the association with recurrent miscarriage through an effect on the ovulatory process.

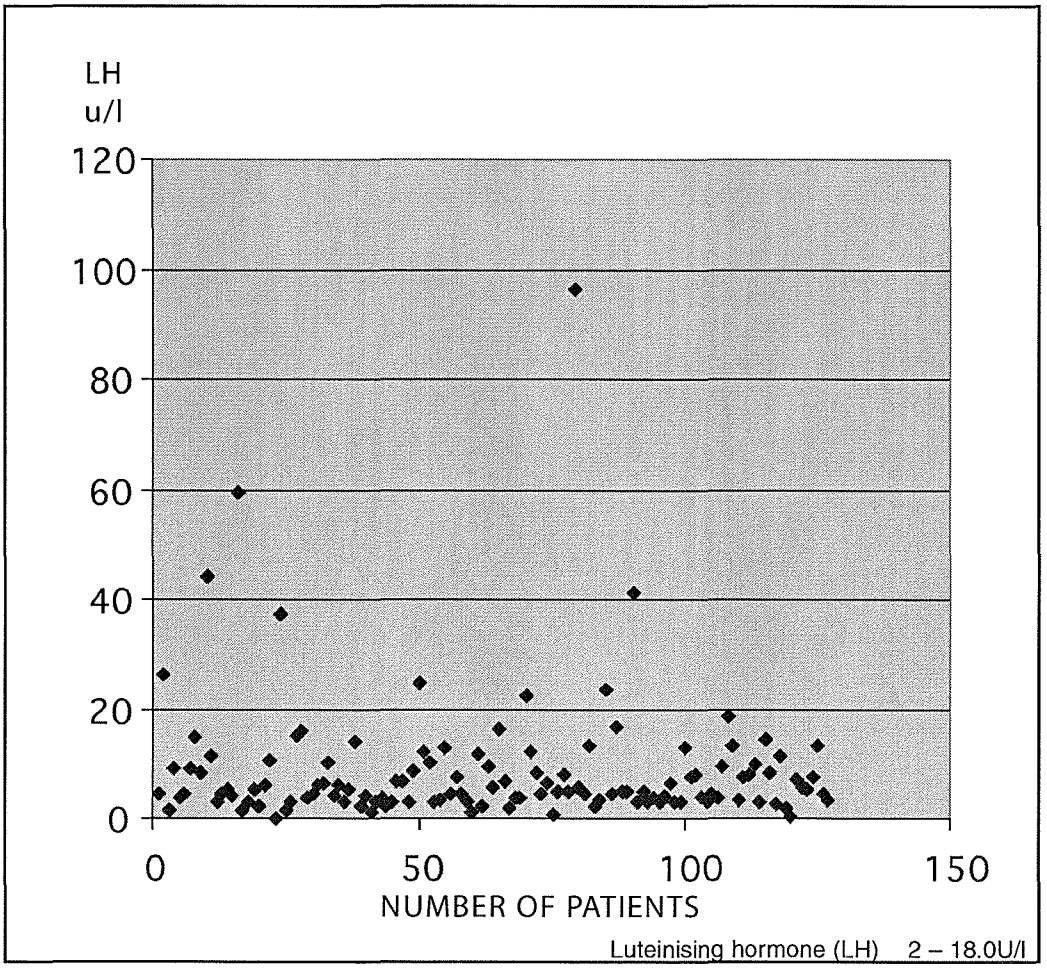


**Fig. 11** The distribution of LH levels in 135 patients referred to the miscarriage clinic

# Serum PROGESTERONE

The mean serum progesterone level was 28.72 nmol\l.

This is considered to be slightly lower than the level indicating an ovulatory cycle.
Two conditions have been associated with recurrent miscarriage and low luteal phase progesterone levels.
The first is luteal phase deficiency and the second is polycystic ovary syndrome.

The mean value is below the 30 nmol level because of the presence of patients with the above conditions. Most of the other patients had normal mid-luteal progesterone levels, especially because of a significant idiopathic group.

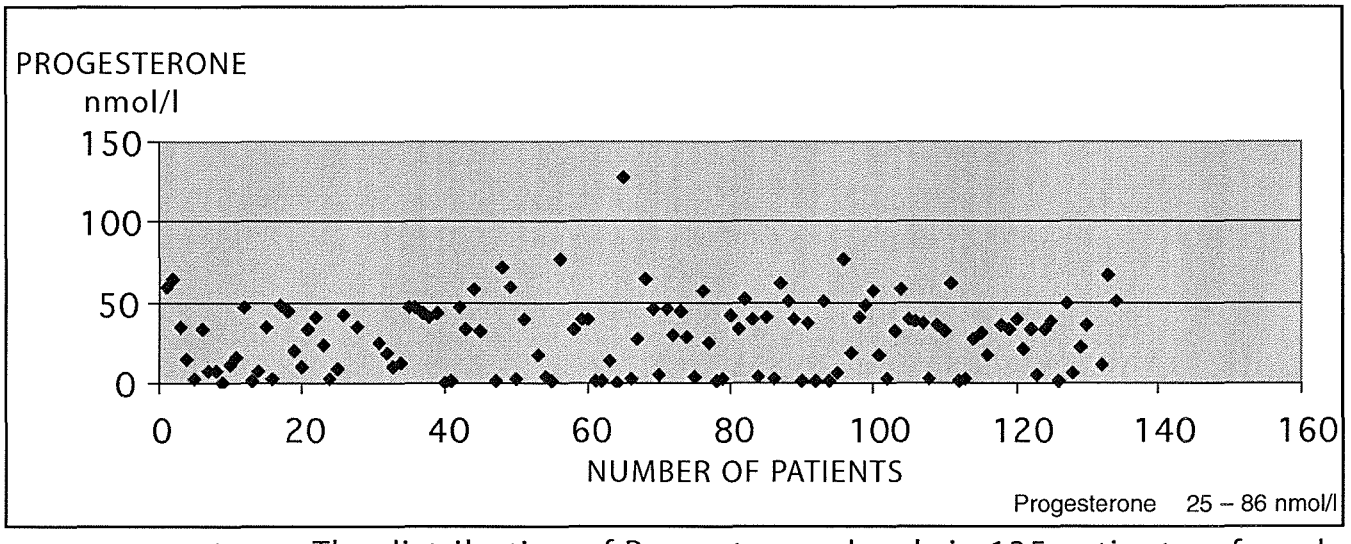


**Fig. 12** The distribution of Progesterone levels in 135 patients referred to the miscarriage clinic

# Serum TESTOSTERONE

The mean serum testosterone level was 6.05, the units being pmol\l.

Hyperandrogenaemia is a feature of polycystic ovarian syndrome.

A scrutiny of the distribution graph for testosterone clearly discriminates this group of patients with levels above the line indicating 10pmol\l.

Some patients had very high testosterone levels and these were found to be resistant to clomiphene therapy and ovulation and conception had to be induced with FSH following down-regulation with gonadotrophin releasing hormone agonists.

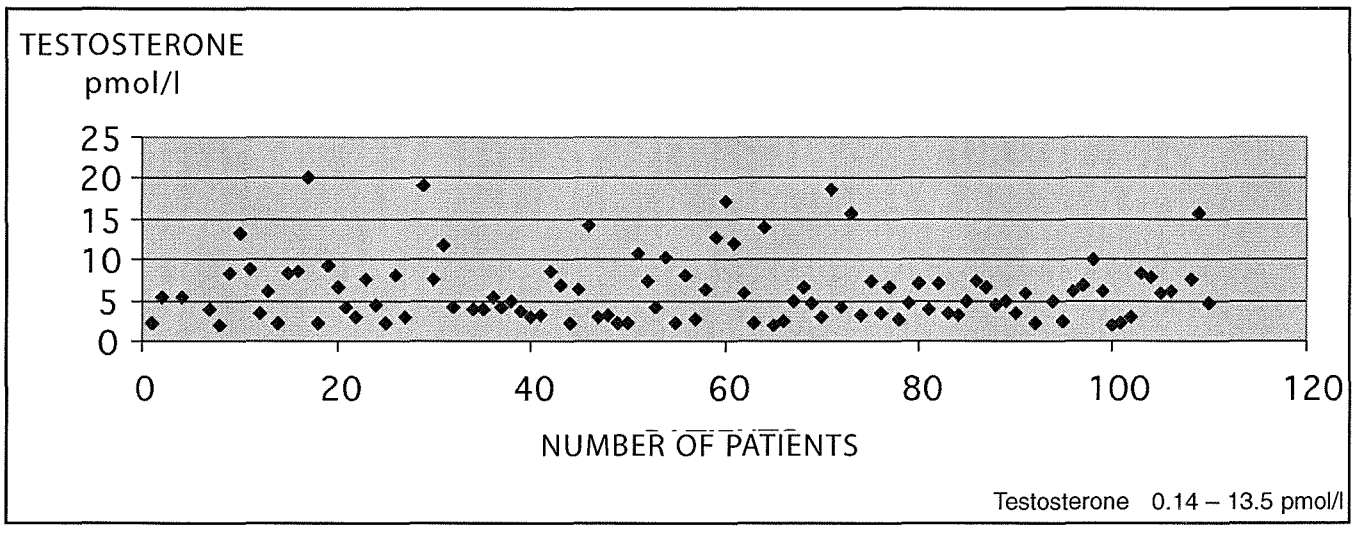


**Fig. 13** The distribution of Testosterone levels in 135 patients referred to the miscarriage clinic

# Serum PROLACTIN

An examination of the serum prolactin levels within this group of patients shows that there was one case of significant hyperprolactinaemia while in five other cases the serum prolactin was raised above normal but below 1000mu/litre.

The mean serum level was 365.35mu\litre which is well within the normal range.

Hyperprolactinaemia is not considered to be a risk factor for miscarriage but is for infertility. Subtle elevations in serum prolcatin levels may accompany polycystic ovarian syndrome and this latter is associated with recurrent miscarriage.

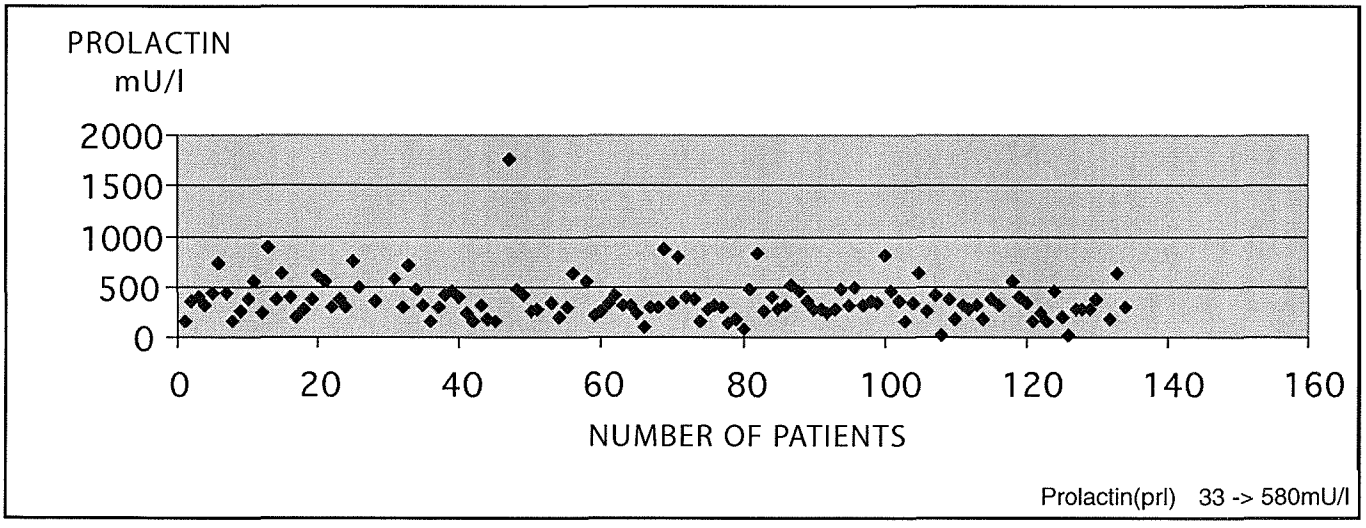


**Fig. 14** The distribution of Prolactin levels in 135 patients referred to the miscarriage clinic

# Serum ESTRADIOL

The serum estradiol levels shows a fairly wide scatter between 0 and 1000 pmol/litre.

The wide scatter is probably due to the fact that patients cycles vary widely and a definite group of patients with oligomenorrhoea was present in the study population, while others reported short cycles.

This means that a mid-luteal phase hormone profile will be ovulatory for some explaining the high readings obtained.

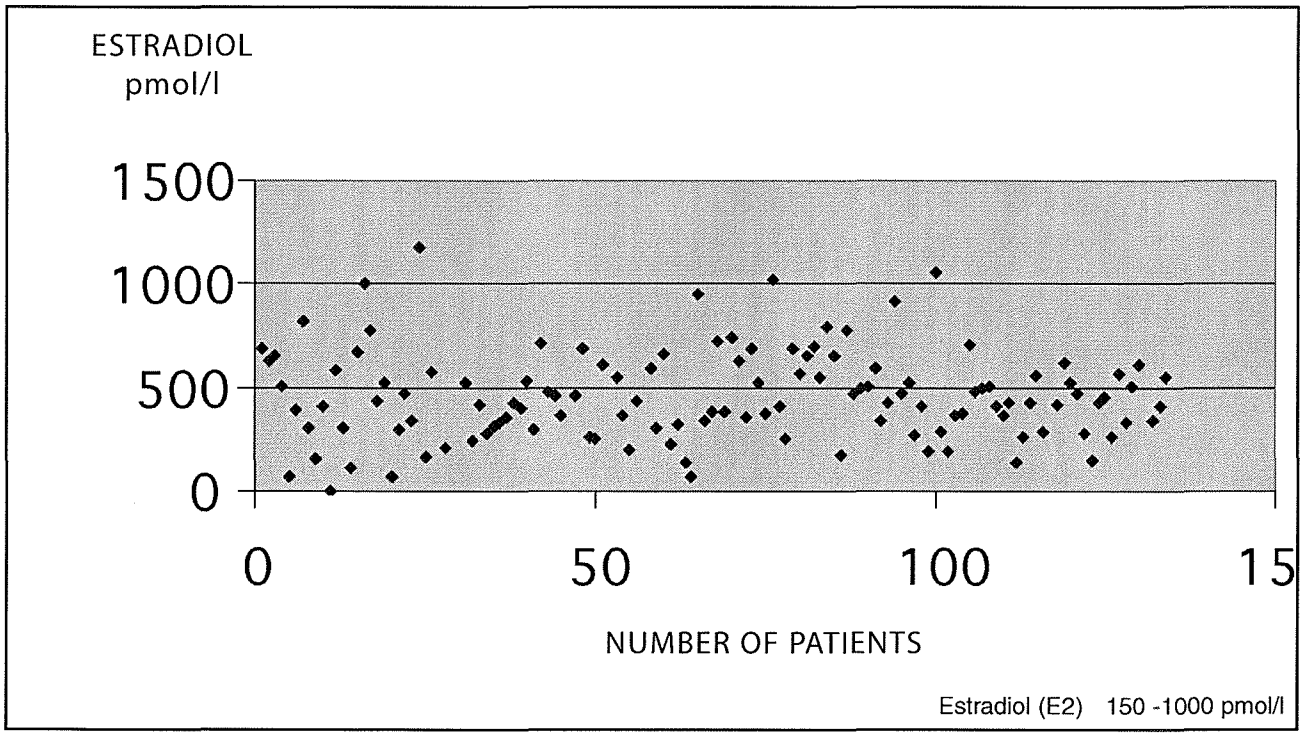


**Fig. 15** The distribution of Estradiol levels in 135 patients referred to the miscarriage clinic

Serum estradiol reflects the presence and degree of maturation of oocytes.

Low pre-conceptual oestradiol levels can only mean poor ovarian function and although progesterone has been widely studied as a factor in miscarriage; low-follicular phase E2 levels have been proposed as a risk factor for recurrent miscarriage in cases with oligomenorrhoea.

# Serum Thyroid Stimulating Hormone

In most cases thyroid function was assessed using a serum TSH assay. Thyroxine levels were only assayed if the serum TSH was abnormal or borderline.

Three cases of hypo-thyroidism (compensated i.e. normal T4 levels) were detected by including this test in the panel of tests for recurrent miscarriage. Another two cases were borderline and did not need treating on subsequent testing.

The mean sTSH level was 2.58 uU/ml.

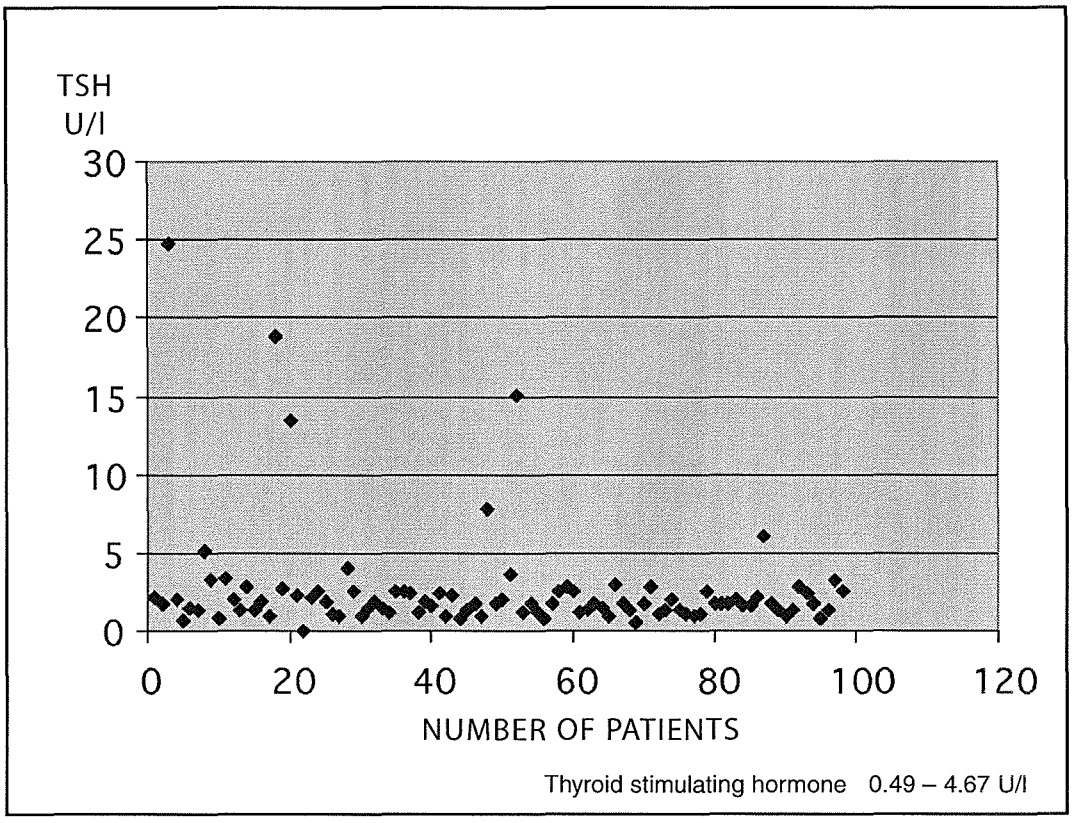


**Fig. 16** The distribution of TSH levels in 135 patients referred to the miscarriage clinic

# Serum THYROXINE

One case of mild hyperthyroidism was diagnosed and referred for management.

All the other patients had normal thyroid function.

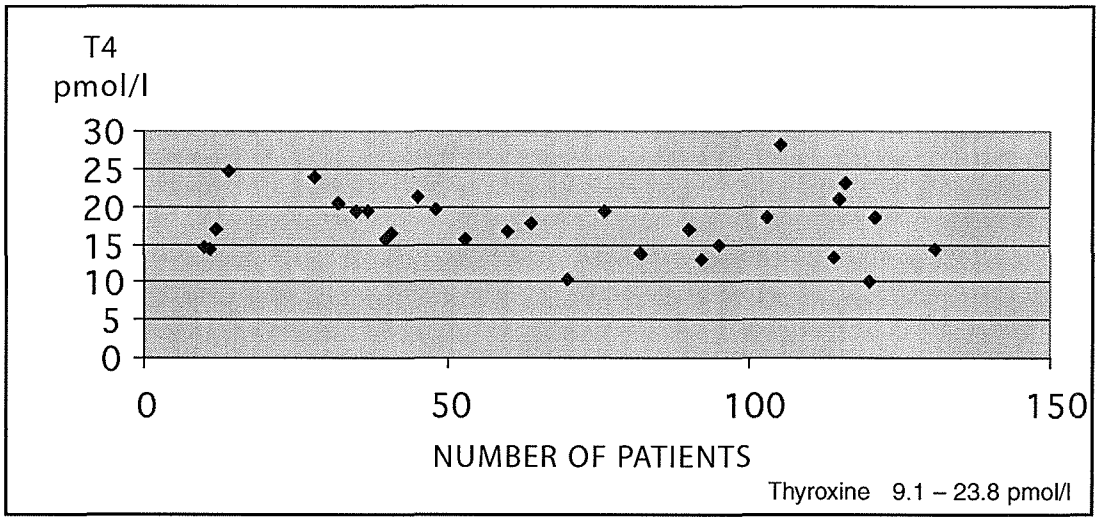


**Fig. 17** The distribution of T4 levels in 135 patients referred to the miscarriage clinic

# THE ORAL GLUCOSE TOLERANCE TEST

The OGTT was recorded as the 2nd hour blood glucose level in mmol/l per test. A glucose load of 75 gms was used. This test is the standard diagnostic test for diabetes mellitus.

The mean 2nd hour blood glucose value was 5.18mmmol/l which is well below the range for impaired glucose tolerance (8-11mmol/l with values above this being diagnostic of diabetes mellitus).

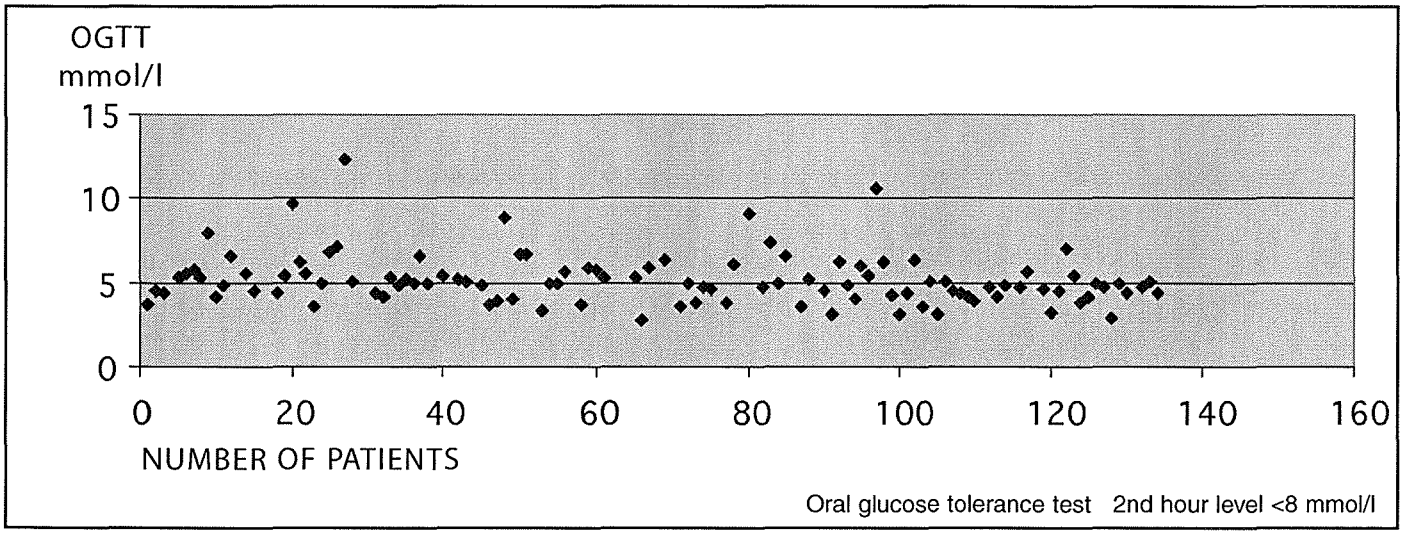


**Fig. 18** The distribution of second hour blood glucose levels (OGTT) in 135 patients referred to the miscarriage clinic

There were four cases of impaired glucose tolerance and two cases of frank diabetes diagnosed from the clinic.

Uncontrolled diabetes is associated with an increased risk of congenital malformation and miscarriage. The role of impaired glucose tolerance in this respect is not clear but proper control before conception is strongly advised.

# RESULTS

## CAUSES AND CLINICAL FEATURES

# RESULTS : CAUSES AND FEATURES OF THE CASES STUDIED

| | $0^{+2}$ | $1^{+2}$ | $0^{+3}$ | $1^{+3}$ | $0^{+>3}$ | $1^{+>3}$ | Total |
|---|---|---|---|---|---|---|---|
| Unexplained | 27 | 15 | 14 | 7 | 2 | 4 | 69 |
| Polycystic ovary syndrome PCOS | 3 | / | 8 | 3 | 1 | / | 14 |
| Luteal phase defect LPD | 5 | / | 2 | / | / | / | 7 |
| Müllerian anomalies | 3 | 1 | 5 | / | 1 | / | 10 |
| Cervical incompetence | 2 | / | 3 | 1 | / | 1 | 7 |
| Anticardiolipin antibody Syndrome | 6 | 1 | 1 | 4 | 1 | 1 | 14 |
| Chromosomal | / | / | 2 | / | / | 1 | 3 |

**Table 4.** Causes Of Recurrent Miscarriage Re Miscarriage History

The largest group in this population studied was the idiopathic group (or unexplained).

They were commonest in the group with only two miscarriages and this may be due to the high prevalence of normal women in this group; women who suffer two recurrent miscarriages simply because of chance.

This group was also well represented in the group of women who had three recurrent miscarriages. In all the unexplained group accounted for over half the cases (51.1%).

The second commonest group was the group with ovulatory dysfunction (21.6%). This comprises the group with polycystic ovary syndrome (15) and luteal phase defects (7). These conditions are poorly represented in the group of women who

had a child before the miscarriages (3 in all). This is because ovulatory dysfunction is associated with both infertility and miscarriage so they are unlikely to be present in women who have had a child.

Congenital anatomical anomalies accounted for 17 cases. The largest group were due to mullerian fusion anomalies (10 cases) while there were 7 cases of cervical incompetence.

In 14 cases there was a high titre of anticardiolipin antibodies reported (these being due to either an elevated IgG or IgM anticardiolipin antibody. In a few case both were raised).
In all cases the positive results related to raised anti-cardiolipin antibody levels. No cases positive for lupus anticoagulant were detected.

| INVESTIGATION : ANTICARDIOLIPIN ANTIBODY | |
|---|---|
| IgM MPL u/ml | IgG GPL u/ml |
| 6 | 20 |
| 16 | 20 |
| 33 | 31 |
| 2 | 36 |
| 10 | 48 |
| 14 | 43 |
| 28 | 5 |
| 34 | 37 |
| 42 | 9 |
| 26 | 5 |
| 34 | 47 |
| 10 | 46 |
| 13 | 35 |
| 10 | 25 |

**Table 5.** IgM and IgG antibody levels in 14 cases of anticardiolipin antibody positive patients

As can be seen from this table both IgM and IgG antibodies can be positive for anti-cardiolipin with, however, IgG being twice as often positive as IgM (3 vs 6 negative results, within the positive group).

This underlines the importance of performing both assays as nine cases were only positive for one of the two antibodies.

## Gynaecological Characteristics vs Miscarriage History

| | $0^{+2}$ | $1^{+2}$ | $0^{+3}$ | $1^{+3}$ | $0^{+>3}$ | $1^{+>3}$ |
|---|---|---|---|---|---|---|
| Menstrual cycle (mean/days) | 30.8 | 27.7 | 29.8 | 31.6 | 28 | 31 |
| Menarche (mean/years) | 12 | 12 | 12 | 12 | 13 | 13 |
| Infertility (no. of patients) | 11 | 2 | 7 | 1 | 1 | 0 |

**Table 6.** The Gynaecological Characteristics of 135 patients referred to the miscarriage clinic distributed by their miscarriage history

The menstrual cycle showed a fairly even distribution with miscarriage history. It was shortest for patients with one child and two miscarriages (27.7 days) and longest for those with one child and three recurrent miscarriages (31.6 days). Both values would be considered to be normal cycles in the general population.

The mean age of the menarche was very stable at 12 to 13 years irrespective of the miscarriage history.

A history of infertility was, however, much commoner in those patients with no living children. Recurrent miscarriage can be a precursor of secondary infertility in some cases especially where ovulatory problems are concerned.
Normal conception rates is a good prognostic factor in recurrent miscarriage.

## Therapeutic Characteristics vs Miscarriage History

| | $0^{+2}$ | $1^{+2}$ | $0^{+3}$ | $1^{+3}$ | $0^{+>3}$ | $1^{+>3}$ |
|---|---|---|---|---|---|---|
| Past O.C.P. use | 8 | 5 | 1 | 2 | 1 | 1 |
| Clomiphene citrate | 15 | 5 | 10 | 5 | 3 | 2 |
| Human menopausal Gonadotrophin (HMG) | 4 | 0 | 3 | 1 | 0 | 0 |

**Table 7.** The Therapeutic Characteristics of 135 patients referred to the miscarriage clinic distributed by their miscarriage history

A history of previous use of the oral contraceptive pill was commonest in patients with two recurrent miscarriages (8) and no children and least common in patients with three or more miscarriages (1). The lack of consistency in these two similar groups supports the idea that this is a coincidence and not because of a causal relationship.

Use of clomiphene citrate was, however, consistently much higher in the patients with no living children; both with two and three recurrent miscarriages. This reflects the increased rates of infertility in these groups.

Again human menopausal gonadotrophin was used in these two groups only with one exception. HMG was used in cases of severe polycystic ovary syndrome resistant to clomiphene with prior down-regulation with gonadotrophin releasing hormone agonists (goserelin by implant).

## Past Gynaecological Conditions

| Conditions | No. of cases | Miscarriage history |
|---|---|---|
| | | |
| Endometriosis | 3 cases | 0+2, 0+3 and 1+3 |
| Menorrhagia | 2 cases | 0+3 and 1+2 |
| Chronic pelvic pain | 1 case | 0+3 |
| Ectopic gestation | 1 case | 0+5 |
| Ovarian cystectomy | 1 case | 0+2 |

**Table 8.** The Past Gynaecological conditions of 135 patients referred to the miscarriage clinic distributed by their miscarriage history

Eight patients presented a past history of gynaecological conditions. The commonest was endometriosis with three cases all having a different miscarriage history, one of them having had a child before.

Endometriosis has been associated with infertility but not with an increased risk of miscarriage once pregnant.

The other gynaecological conditions were menorrhagia, chronic pelvic pain, an ectopic pregnancy and one patient had an ovarian cystectomy in pregnancy which she subsequently miscarried.

## Chronic Medical Conditions

| Conditions | No of cases | Miscarriage history |
|---|---|---|
| Colitis | 1 case | 0+2 |
| Hepatitis | 2 cases | 0+2,0+2 |
| Asthma | 4 cases | 0+3, 1+2, 0+2, 0+2 |
| Poliomyelitis | 1 case | 0+3 |
| Hypertension | 3 cases | 0+2, 0+3, 0+3 |
| Hirsutism | 1 case | 0+2 |
| Malaria- past history | 1 case | 0+4 |
| IDDM | 1 case | 1+3 |
| Prolactinoma | 1 case | 1+2 |
| Peptic ulcer | 1 case | 0+3 |
| DVT | 2 cases | 0+2, 0+7 |
| Hypo thyrodism | 2 cases | 1+2, 0+3 |
| Adult Polycystic kidney disease | 1 case | 0+4 |
| Glomerulo nephritis | 1 case | 0+2 |
| Duplex kidney | 1 case | 0+2 |
| Rheumatoid arthritis | 1 case | 1+2 |

**Table 9.** The Chronic Medical conditions of 135 patients referred to the miscarriage clinic.

The most common medical condition in this group of patients was bronchial asthma. This is not usually considered to be a risk factor for miscarriage but the second commonest, hypertension is, especially if not well controlled.

The patient with adult polycystic kidney disease suffered from severe hypertension which was difficult to control. She also had early signs of renal failure with chronic anaemia.

Both cases who reported an episode of deep vein thrombosis proved to be cases of antiphospholipid antibody syndrome.

Most chronic medical conditions should not be a threat to pregnancy but control is essential.

# RESULTS

## OUTCOME OF THE CASES STUDIED

## Key to abbreviations :

| | |
|---|---|
| NAD | no abnormality detected |
| LDA | low dose aspirin |
| NO R | no treatment |
| PRED | prednisolone |
| ACA+ve | anticadiolipin antibody positive |
| LPD | luteal phase defect |
| PCOS | polycystic ovary syndrome |
| TOCO | protocol of tocolytic drugs |
| GnRHA | gonadotrophin releasing hormone agonist |
| C&T | cerclage and tocolysis |
| APS | antiphospholipid antibody syndrome |
| HCG | human chorionic gonadotrophin |
| HMG | human menopausal gonadotrophin |
| IDDM | insulin-dependent diabetes mellitus |

# RESULTS AND OUTCOME PER INDIVIDUAL PATIENT

**GROUP** **1**
**PATIENTS WITH TWO CONSECUTIVE MISCARRIAGES AND NULLIPAROUS**

| CODE NO | FEATURES | OUTCOME |
|---|---|---|
| 017 | NAD; NO R | MISCARRIAGE |
| 094 | NAD; NO R | MISCARRIAGE |
| 095 | NAD; NO R | MISCARRIAGE |
| 090 | NAD | NO PREGNANCIES |
| 133 | NAD | NO PREGNANCIES |
| 019 | NAD; NO R | LIVE BABY |
| 040 | NAD; NO R | LIVE BABY |
| 063 | NAD; NO R | LIVE BABY |
| 072 | NAD; NO R | LIVE BABY |
| 006 | NAD; NO R | LIVE BABY |
| 100 | NAD; NO R | LIVE BABY |
| 119 | NAD; NO R | LIVE BABY |
| 132 | NAD; NO R | LIVE BABY |
| 129 | NAD; NO R | LIVE BABY |
| 003 | NAD; LDA | LIVE BABY |
| 004 | NAD; LDA | LIVE BABY |
| 030 | NAD; LDA | LIVE BABY |
| 060 | NAD; LDA | LIVE BABY |
| 076 | NAD; LDA | LIVE BABY |
| 077 | NAD; LDA | LIVE BABY |
| 078 | NAD; LDA | LIVE BABY |
| 088 | NAD; LDA | LIVE BABY |
| 073 | NAD; LDA | LIVE BABY |
| 101 | NAD; LDA | LIVE BABY |
| 103 | NAD; LDA | LIVE BABY |

| | | |
|---|---|---|
| 118 | NAD; LDA | LIVE BABY |
| 011 | NAD; PRED/LDA | LIVE BABY |
| 069 | ACA+VE; LDA | LIVE BABY |
| 134 | ACA+VE;LDA | LIVE BABY |
| 089 | ACA+VE; HEP/LDA | LIVE BABY |
| 009 | ACA+VE; PRED/LDA | LIVE BABY |
| 053 | APS; WARFARIN | LIVE BABY |
| 062 | SLE | NO PREGNANCY |
| 117 | LPD; CLOMID | LIVE BABY |
| 102 | LPD; CLOMID | LIVE BABY |
| 022 | LPD;CLOMID | LIVE BABY |
| 021 | LPD | NO PREGNANCY |
| 054 | PCOS; NO TREATMENT | L IVE BABY |
| 016 | PCOS; | NO PREGNANCY |
| 051 | PCOS; CLOMID | MISCARRIAGE |
| 074 | BICORNUATE;TOCO | LIVE BABY |
| 052 | BICORNUATE;TOCO | LIVE BABY |
| 057 | BICORNUATE; TOCO | LIVE BABY |
| 070 | BICORNUATE;TOCO | LIVE BABY |
| 086 | OLIGOMENORRHOEA | NO PREGNANCIES |
| 005 | CERCLAGE | LIVE BABY |
| 131 | CERCLAGE | LIVE BABY |
| 120 | CERCLAGE | MISCARRAIGE |

## GROUP B; THREE CONSECUTIVE MISCARRIAGES AND NULLIPAROUS

| CODE NO | FEATURES | OUTCOME |
|---|---|---|
| 007 | INVERSION | LIVE BABY |
| 082 | TRANSLOCATION | LIVE BABY |
| 013 | NAD; NO R | MISCARRIAGE |
| 014 | NAD; NO R | LIVE BABY |
| 027 | NAD; NO R | LIVE BABY |
| 027 | NAD; NO R | LIVE BABY |
| 027 | NAD; NO R | LIVE BABY |
| 055 | NAD; NO R | LIVE BABY |
| 121 | NAD; NO R | LIVE BABY |
| 010 | NAD | NO PREGNANCIES |
| 065 | NAD | NO PREGNANCIES |
| 065 | NAD | NO PREGNANCIES |
| 092 | NAD | NO PREGNANCIES |
| 091 | NAD; LDA/PRED | LIVE BABY |
| 031 | NAD; LDA/PRED | LIVE BABY |
| 093 | NAD; LDA/PRED | LIVE BABY |
| 032 | CERCLAGE | LIVE BABY |
| 084 | CERCLAGE | NO PREGNANICES |
| 034 | ACA+VE; LDA/HEP | MISCARRIAGE |
| 020 | ACA+VE; HEP/LDA | LIVE BABY |
| 080 | ACA+VE | NO PREGNANCIES |
| 041 | ANTI-THYROID;LDA/HEP | LIVE BABY |
| 023 | PCOS; CLOMID | MISCARRIAGE |
| 04 | PCOS; CLOMID | MISCARRAIGE |
| 085 | PCOS; GnRHA/HMG | LIVE BABY |

| | | |
|---|---|---|
| 012 | PCOS GnRHA/HMG | LIVE BABY |
| 064 | BICORNUATE; TOCO | MISCARRIAGE |
| 104 | BICORNUATE;C&T | MISCARRIAGE |
| 123 | BICORNUATE; C&T | LIVE BABY |
| 083 | BICORNUATE; C&T | LIVE BABY |
| 10 | FIBROID | MISCARRIAGE |

**GROUP C; FOUR CONSECUTIVE MISCARRIAGES AND NULLIPAROUS**

| | | |
|---|---|---|
| 049 | DIABETES MELLITUS | NO PREGNANCIES |
| 098 | DIABETES MELLITUS | NO PREGNANCIES |
| 059 | METROPLASTY | LIVE BABY |
| 116 | NAD | NO PREGNANCIES |
| 125 | APS | NO PREGNANCIES |

**GROUP D ; TWO CONSECUTIVE MISCARRAIGES AND ONE LIVING CHILD**

| | | |
|---|---|---|
| 043 | NAD ;NO R | NO PREGNANCIES |
| 075 | NAD; NO R | NO PREGNANCIES |
| 127 | NAD; NO R | NO PREGNANCIES |
| 044 | NAD ;NO R | LIVE BABY |
| 046 | NAD; NO R | LIVE BABY |
| 047 | NAD; NO R | LIVE BABY |
| 061 | NAD; NO R | LIVE BABY |
| 061 | NAD; NO R | LIVE BABY |
| 128 | NAD; NO R | LIVE BABY |
| 106 | NAD; NO R | LIVE BABY |
| 071 | NAD; NO R | MISCARRAIGE |
| 108 | NAD; NO R | MISCARRIAGE |
| 110 | NAD; NO R | MISCARRIAGE |

| | | |
|---|---|---|
| 058 | NAD; LDA | LIVE BABY |
| 087 | NAD; LDA | LIVE BABY |
| 067 | NAD; LDA/DUPHASTON | MISCARRIAGE |
| 037 | NAD;LDA&DUPHASTON | MISCARRIAGE |
| 112 | NAD; DUPHASTON | MISCARRIAGE |
| 036 | PCOS/CLOMID&HCG | MISCARRIAGE |
| 036 | PCOS/CLOMID&HCG | MISCARRIAGE |
| 045 | PCOS; NO R | LIVE BABY |
| 045 | PCOS; NO R | LIVE BABY |
| 099 | BICORNUATE; NO R | LIVE BABY |
| 048 | PROLACTINAEMIA | NO PREGNANCIES |
| 127 | hypothalamic amenorrhoea | NO PREGNANCIES |

**GROUP E; THREE CONSECUTIVE MISCARRAIGES AND ONE LIVING CHILD**

| | | |
|---|---|---|
| 109 | NAD; NO R | MISCARRAIGE |
| 135 | NAD; NO R | MISCARRAIGE |
| 068 | NAD; LDA/DUPHASTON | LIVE BABY |
| 015 | PCOS | NO PREGNANCIES |
| 035 | PCOS | NO PREGNANCIES |
| 096 | PCOS | NO PREGNANCIES |
| 056 | PCOS; ZOLADEX/HMG | MISCARRIAGE |
| 024 | LPD; CLOMID/DUPHASTON | LIVE BABY |
| 025 | LPD; CLOMID/DUPHASTON | MISCARRIAGE |
| 130 | LPD; CLOMID | LIVE BABY |
| 050 | ACA+VE | NO PREGNANCIES |
| 038 | ACA+VE; LDA | LIVE BABY |
| 066 | ACA+VE; LDA/HEPARIN | LIVE BABY |
| 107 | ACA+VE; LDA/HEPARIN | LIVE BABY |
| 029 | CERCLAGE | LIVE BABY |

| | | |
|---|---|---|
| 026 | HYPOTHYROID | MISCARRAIGE |
| 028 | IDDM | MISCARRAIGE |
| 018 | TRANSLOCATION | NO PREGNANCIES |

**GROUP F; FOUR CONSECUTIVE MISCARRIAGES AND ONE LIVING CHILD**

| | | |
|---|---|---|
| 033 | NAD; LDA/DUPHASTON | LIVE BABY |
| 097 | NAD ; NO R | NO PREGNANAICES |
| 039 | ACA+VE; LDA | MISCARRIAGE |

**GROUP G; TWO CONSECUTIVE AND TWO LIVING CHILDREN**

| | | |
|---|---|---|
| 126 | NAD NO R | LIVE BABY |
| 002 | NAD; NO R | LIVE BABY |
| 081 | NAD; NO | LIVE BABY |
| 111 | NAD; NO R | NO PREGNANCIES |
| 113 | NAD; NO R | NO PREGNANCIES |
| 124 | CERCLAGE; TOCOLYSIS | LIVE BABY |

# RESULTS

## OUTCOME RECORDED PER TREATMENT PROTOCOL

# RESULTS: OUTCOME vs TREATMENT PROTOCOL

## UNEXPLAINED OR NO ABNORMALITY DETECTED

**1. No Treatment Prescribed**

**Number of patients: 47**

9 miscarriages

25 babies

13 no pregnancies

**2. Low Dose Aspirin**

**Number of patients:** 14

14 babies

**3. Low dose aspirin and prednisolone**

**Number of patients:** 4

4 babies

**4. Low dose aspirin and duphaston**

**Number of patients:** 4

2 miscarriages

2 babies

**TOTAL 69**

**11 miscarriages**

**45 babies**

**13 no pregnancies**

# ENDOCRINE DEFECTS

## LUTEAL PHASE DEFECT

### 1. No Treatment

**Number of patients:** 1 case

no pregnancy

### 2. Clomiphene/Duphaston/Human Chorionic Gonadotrophin

**Number of patients:** 6

5 babies

1 miscarriage

## POLYCYSTIC OVARY SYNDROME

### 1. No Treatment

**Number of patients:** 7

3 babies

1 miscarriage

3 no pregnancies

### 2. Clomiphene/Duphaston/HCG

**Number of patients:** 5

5 miscarriages

### 3. Goserelin and HMG

**Number of patients:** 3

2 babies

1 miscarriage

**TOTAL 22**

**10 babies**

**8 miscarriages**

**4 no pregnancies**

# ANATOMICAL ABNORMALITIES

## CERVICAL INCOMPETENCE

**All treated with a cervical cerclage:**

**Number of patients:** 7

5 babies

2 miscarriages

## BICORNUATE UTERUS

**1. Tocolysis +\- cerclage**

**Number of patients:** 9

7 babies

2 miscarriages

**2. Metroplasty**

**Number of patients:** 1 case

1 baby

**TOTAL 17**

**13 babies**

**4 miscarriages**

## ANTICARDIOLIPIN ANTIBODY POSITIVE

**1. No Treatment**

**Number of patients:** 2

no pregnancies

**2. Low dose Aspirin**

**Number of patients:** 4

3 babies

1 miscarriage

**3. LDA\Prednisolone**

**Number of patients:** 1

1 baby

**4. LDA\Heparin**

**Number of patients:** 5

4 babies

1 miscarriage

## ANTIPHOSPHOLIPID ANTIBODY SYNDROME

**WARFARIN**

**Number of patients:** 2

1 baby

1 miscarraige

**TOTAL 14**

**9 babies**

**3 miscarriages**

**2 no pregnancies**

## OTHERS

Antithyroid Antibodies (para 0+3); live child on LDA\Heparin

Uterine fibroid (para 0+3) ; another miscarraige

Insulin dependent diabetes mellitus ; 3 cases. One had a miscarriage and two did not conceive again.

Systemic lupus erythematosus : 2 cases. One live baby and one did not conceive again

Hypothyroidism ; suffered another miscarriage
Prolactinoma ; did not conceive again

Premature Ovarian Failure ; did not conceive again

Parental chromosomal abnormalities ; 3 cases. Two babies and one did not conceive further.

**OUTCOME TOTAL 135**
**81 babies**
**29 miscarriages**
**25 no pregnancies**

# RESULTS

## STATISTICAL ANALYSIS

# STATISTICAL ANALYSIS

All the variables that have been studied were subjected to a logistic regression analysis in order to ascertain if any of the variables achieved statistical significance in the population studied.

A live birth was taken to be the event against which all the information for each patient studied, each as individual variables were studied.

Both a univariate (the results are not adjusted for the effect of the individual variables on each other) and a multivariate analysis (where there is such an adjustment).
Variables could be either zero to one or they could be continuous.

The data was analysed using Intercooled Stata Version 7 (STATA Corporation Texas USA).

The results are illustrated in tables 10 and 11.

# TABLE 10 :
# UNIVARIATE ANALYSIS OF THE CLINICAL CHARACTERISTICS RECORDED

| CHARACTERISTICS | CATEGORIES | ODDS RATIO | INTERVAL | P-VALUE |
|---|---|---|---|---|
| AGE | / | 0.91 | 0.85 – 0.97 | 0.005 |
| CYCLE LENGTH | / | 0.95 | 0.90 – 1.01 | 0.100 |
| CLOMID (USE OF) | NOT USED<br>USED | 1.0<br>0.30 | 0.135 – 0.67 | 0.003 |
| FSH (LEVEL) | | 0.86 | 0.78 – 0.96 | 0.006 |
| ULTRASOUND 1 | NORMAL<br>ABNORMAL | 1<br>1.44 | 0.20 – 10.6 | 0.72 |
| ULTRASOUND 2 | NORMAL<br>ABNORMAL | 1<br>3.84 | 0.96 – 15.3 | 0.050 |
| ULTRASOUND 3 | NORMAL<br>ABNORMAL | 1<br>0.72 | 0.63 – 8.20 | 0.792 |

KEY : ULTRASOUND 0 NORMAL

ULTRASOUND 1 POLYCYSTIC OVARY SYNDROME

ULTRASOUND 2 CONGENITAL UTERINE ANOMALIES

ULTRASOUND 3 FIBROIDS

# TABLE 11
# MULTIVARIATE ANALYSIS OF CLINICAL CHARACTERISTICS RECORDED

| CHARACTERISTICS | CATEGORIES | ODDS RATIO | INTERVAL | P-VALUE |
|---|---|---|---|---|
| AGE | / | 0.89 | 0.82 – 0.97 | 0.007 |
| CYCLE LENGTH | / | 0.92 | 0.10 – 0.85 | 0.044 |
| CLOMID (USE OF) | NOT USE<br>USE | 1.0<br>0.29 | 0.80 – 0.10 | 0.050 |
| FSH (LEVEL) | / | 0.894 | 0.80 – 1.0 | 0.050 |
| ULTRASOUND 1 | NORMAL<br>ABNORMAL | 1<br>4.03 | 0.45 – 36.4 | 0.214 |
| ULTRASOUND 2 | NORMAL<br>ABNORMAL | 1<br>8.15 | 1.51 – 43.9 | 0.015 |
| ULTRASOUND 3 | NORMAL<br>ABNORMAL | 1<br>1.08 | 060 – 19.4 | 0.957 |

**KEY : as on previous page**

The variables which resulted in a significant odds ratio were :

- age
- use of clomiphene citrate
- follicle stimulating hormone level
- cycle length
- an ultrasound report of a congenital uterine anomaly

The variables age, use of clomiphene citrate and level of follicle stimulating hormone, all achieved statistical significance with p-values < 0.005, <0.003, <0.006 in a univariate analysis. Level of FSH did not maintain this level of significance in a multivariate analysis.

Cycle length and ultrasound examinations reporting a congenital abnormality of the uterus reported p-values of 0.1 and 0.05 respectively.
While these are not strictly speaking statistically significant they may indicate factors which have a bearing on the outcome of recurrent miscarriage.

Of note is the fact that age was statistically significant when analysed both in a univariate and multivariate method.

Reference to these results will be made in the general discussion to follow.

# DISCUSSION

# CHAPTER I

The cause which had the largest number of patients in this study group was the **idiopathic** or **unexplained group.**

This group represented just over half the patients studied (51.1%) with a total of 69 cases.
The majority of these 29 (68.1%) were in the group of patients who had suffered two recurrent miscarriages.

This result would confirm the notion that most patients with only two recurrent miscarriages are normal and have a good prognosis.
This is in fact confirmed by the results obtained. Out of 69 patients 45 babies were recorded in total representing a success rate of 65.2%.
This, however, meant that 24 couples were still childless at the end of the study period. This fact emphasizes that the idiopathic group deserves as much attention as all the rest.

It has been calculated that between 1 to 2% of fertile women will experience recurring pregnancy loss (6).
Protocols for the investigation of recurrent miscarriage should identify a cause in about 50% of cases ( 7 ).
Studies have shown that up to 50% of all (including spontaneous) miscarriages are due to chromosomal defects, which

leaves another fifty per cent due to either unknown or preventable disease.

Failure to make a diagnosis as to the cause of recurrent miscarriage has led to the title '**unexplained recurrent miscarriage'.** This situation is very different from a similar situation like, for example, unexplained infertility. In the latter situation some mechanism, as yet unexplained must be operating to prevent a couple from conceiving.

In recurrent miscarriage, different causes may operate in successive miscarriages leading to the same conclusion but not due to a recurrent cause (8).

The various treatment options used were either no treatment prescribed, low dose aspirin, low dose aspirin and prednisolone (now no longer in use) and low dose aspirin and dydrogesterone.

All these treatment protocols are empirical at this present time and the various drugs used are discussed individually later on (see section on autoimmune causes).

Low-dose aspirin either alone or in combination with prednisolone was associated with a 100% positive outcome, but, of course, the numbers are small (18 patients in all).

When combined with dydrogesterone, in four cases, the results were not so good and only 50% of the patients achieved a live healthy baby.

A significant placebo effect has also been reported in studies on the use of progesterone (9) and immunization protocols (10).

This group of patients has particular characteristics because of this feature.

In all controlled studies on this group of patients a significant placebo effect associated with the provision of effective emotional support in the form of a dedicated miscarriage clinic and an early pregnancy assessment unit has been consistent.

Low-dose aspirin is thought to positively influence throphoblastic/uterine circulation which may be affected by adverse immunological/thrombophilic factors as yet undetectable by present knowledge.

Progesterone is increasingly being shown to be important as a modulator of the immunological response in pregnancy and should therefore also be helpful in these circumstances (97).

No real conclusions can be drawn from four patients treated with a combination of aspirin and dydrogesterone and the problem is further compounded by the fact that the assumption that the idiopathic group is as yet unresolved is, of course not true in many cases.

There is no reason why many, if not most, patients with idiopathic recurrent miscarriage would not be perfectly normal. The explanation into their miscarriages would be a series of spontaneous miscarriages because of the well-documented

chromosomal aberrations which occur spontaneously in this group. The prognosis in this group of patients is considered to be favourable (22).

All the patients in this group received the extra care and support which was available through direct access to services via the clinic.

The group of patients who opted not to have any of the treatment protocols did not do as well as the other groups. 25 babies were reported from this group (ie 53.1%, just over half), but 13 couples from this group did not conceive again for various reasons.

That figure should therefore be adjusted to 73.5 %.

This result is consistent with the overall good prognosis of recurrent miscarriage due to unexplained causes. It is also consistent with the positive results reported with supportive care alone, of which, an early ultrasound scan forms an important part.

The role of emotional support in recurrent miscarriage was pioneered by by Stray-Pedersen & Stray-Pedersen in 1984 (11).

In this study a diagnostic screening program was applied to 195 couples with a history of habitual abortion (ie three or more consecutive abortions). Abnormalities were identified in 110 (56%) of the couples. The authors noted that patients with primary habitual abortion and second-trimester miscar-

riage were more likely to have a positive diagnosis than their counter-parts.

They described an 'Unknown etiology' group. This represented 43.6% of their group. In this group first trimester losses were significantly more frequent than second-trimester losses. There were 61 conceptions in this group and thirty-seven were selected for 'tender loving care'. In this group 32 women (86%) carried their pregnancies to term and delivered normal, healthy babies while in the group who received standard antenatal care, only 33% had successful pregnancies. This difference in outcome was found to be highly significant.

In a later trial Liddell et al (12) randomised a group of patients to receive formal emotional support and compared it to a group who received routine care. Again the group receiving 'tender loving care' achieved a successful pregnancy rate of 86%. This figure is comparable to the miscarriage rate of clinically recognisable pregnancies.

The authors pointed out that it is clear that in a number of cases of recurrent miscarriage, this is due to a repeated random event and there should therefore be a spontaneous cure rate.

In another study (13) an 80% successful pregnancy rate was observed after offering no active treatment to 21 women with a history of recurrent miscarriage.

**This degree of success after formal emotional support indicates a therapeutic effect of this approach.**

The experience of repeated miscarriage commonly results in a very marked stress reaction when the woman becomes pregnant again. This is characterised by general tension, weeping, fear of going to the toilet or of examining underwear in case of the presence of bleeding. There is an obsessional attention to the presence of pregnancy symptoms, extreme anxiety over the presence of abdominal pain, discharge and avoidance of other pregnant women or discussing the pregnancy with anyone.

Often there are panic attacks at the gestation of previous miscarriages.

Stress has been linked to premature labour (14).

Miscarriage, intrauterine growth retardation, premature labour may be all part of one pathological process (15,16 ) and if stress can influence premature labour then it may also influence miscarriage. Liddell et al propose that this may be due to either excessive irritability of the uterus or alteration in pelvic blood flow.

In a study on the protocol for the investigation of recurrent miscarriage Clifford et al (22) also noted that the prognosis for the patients in whom no cause could be identified was good. They also observed that patients under the age of 37 years were twice more likely to be successful in a subsequent

pregnancy than patients over the age of 37 years. It is noteworthy that age was one of the variables which was found to be statistically significant in terms of an eventual live birth within the group of patients studied.

There has been concern that the incidence of unexplained recurrent miscarriage may be dependent on the definition i.e. two or three miscarriages. In a study on mid-trimester loss (17) where two successive losses was considered a justifiable entry critierion the incidence was still 50%. In our own population with two miscarriages this was also the case.

Bulletti et al (18) emphasise that the modern social habit is to conceive at a later age; waiting for a third miscarriage prior to investigating may seriously compromise the patients chances to have a child. When the resources are available it is reasonable to investigate and treat after two miscarriages.

The role of ultrasound in 'tender loving care' is considered essential (19).

The appearance of fetal cardiac activity is an important observation both to clinicians and patients. In this study the fetal loss rate was 3% after the detection of fetal cardiac activity. This is better than most other reports in that among women with recurrent pregnancy loss the presence of a live embryo detected by first-trimester ultrasonography is not as encourag-

ing as in normal pregnant women (20).

In the latter study approximately 75% of pregnancies in RPL(recurrent pregnancy loss) will have a live embryo on ultrasound and although the presence of fetal cardiac activity is reassuring in the normal population (less than 4% will go on to miscarry) in RPL the rate of pregnancy loss is four to five times higher.

In a study reported on 302 patients diagnosed as unexplained recurrent aborters (19), 222 had a subsequent pregnancy and of these 75% had a successful outcome, beyond 24 weeks. In this study no difference was noted in outcome between those who had had two and those who had had three miscarriages. There was also no statistical difference between primary and secondary aborters. It was also clear using a survival curve that most miscarriages(89%) in this group occurred between 6 and 8 weeks gestation; 78% without the detection of a fetal heart.

Another important factor which influenced outcome in successive pregnancies was increasing maternal age. In unexplained recurrent miscarriage the spontaneous cure rate referred to above will be affected by increasing maternal age. This is a consistent theme referred to in other studies and cannot be over-emphasised when counselling a patient.

**Age,** as an independent factor proved to be statistically signif-

icant in relation to eventual outcome; the older the patient the more likely she was to suffer a negative outcome.

In a study on maternal age and fetal loss Andersen et al (21 ) noted an increasing risk above the age of 30 years, while at 42 years, more than half of all pregnancies resulted in spontaneous abortion, ectopic pregnancy or stillbirth.

A third study on the outcome of unexplained recurrent miscarriage managed by a dedicated clinic using supportive care alone again re-iterated the excellent results that can be achieved. In the study by Clifford et al (22) 70 to 80 % of women achieved a livebirth in a subsequent pregnancy. Again it was noted that the rate of miscarriage rose sharply after the age of 40 years.
The number of previous miscarriages is considered to be a risk factor for future pregnancy loss (23, 24 ) but in this study even after six miscarriages >45% of women achieved a livebirth without treatment.

The conclusion from these studies is that women in whom no cause can be found for the recurrent miscarriages can be encouraged since the outcome is likely to be a positive one.
Reference to the RCOG Clinical Greentop Guidelines on Recurrent Miscarriage (revised in May 2003) underlines this conclusion ; ' women with unexplained recurrent miscarriage

have an excellent prognosis for future pregnancy outcome without pharmacological intervention if offered supportive care alone in the setting of a dedicated pregnancy assessment unit'.

Early pregnancy support is time-consuming and costly, both in terms of staff and equipment but these results make the availability of one a must for any University Hospital.

# CHAPTER II

The second most important group, numerically speaking, was the endocrine group.

This is represented by the Polycystic Ovary Syndrome and the Luteal Phase Defect. Both conditions are associated with ovulatory dysfunction and call be collectively described as endocrine conditions associated with ovulatory dysfunction. There was a total of 22 patients in this group, 15 cases of Polycystic Ovary Syndrome and 7 cases of Luteal Phase defect. This makes Polycystic Ovary Syndrome the most common positive diagnosis in this group of 135 patients with recurrent miscarriage; an incidence of 11.1%. This is lower than the average reported incidence of PCOS in infertility clinics (20%) and would imply that it is more important as a cause of infertility than miscarriage.

## LUTEAL PHASE DEFECTS

The **luteal phase** of the menstrual cycle is that part of the cycle which commences with ovulation and ends with the onset of menstruation.

It is the LH surge which triggers the changes which culminate

in ovulation, but it also stimulates the production of progesterone by inhibiting the aromatizing activity of the granulosa cells diverting them instead to the production of progesterone. The follicle which ruptures is converted to a corpus luteum by the development of luteal cells which contain a characteristic yellow pigment (lutein). The luteal cells secrete 17beta-hydroxyprogesterone, progesterone and oestrogens. It is also know to secrete peptide hormones.

Endocrine support of the corpus luteum is by luteinising hormone (LH) which can be taken over by human chorionic gonadotrophin in the event of a pregnancy. If there is no pregnancy the corpus luteum begins to degenerate about day ten of the cycle, and will lead to the onset of menstruation.

In 1973 Csapo et al showed that removal of the corpus luteum from pregnant ewes, inevitably lead to miscarriage. This led to the understanding of the importance of progesterone support of the early pregnancy, when patients subjected to the same procedure and treated with progesterone avoided miscarriage(25) .

The condition **luteal phase deficiency or defect (LPD),** still, however, constitutes a controversy in Gynaecology. It is thought to result from insufficient progesterone production by the ovary post-ovulation or from an inadequate progesterone effect on the endometrium (26).

Anovulation is an important cause of luteal phase defects. In this case, however, pregnancy will not occur and therefore in the context of recurrent miscarriage this will not be considered. It is oestrogen secreted during the follicular phase which stimulates the endometrium to produce progesterone receptors. Therefore if oestrogen production during the follicular phase is inadequate, it is likely that this will lead to a luteal phase defect either through inadequate production of progesterone or an inadequate effect of progesterone on the endometrium because of a progesterone receptor defect.

A study (27) showed that women with delayed endometrial development in the luteal phase had significantly lower follicle stimulating hormone in the follicular phase of the same cycle. However Li et al (28) found that in ovulatory cycles there was no significant difference in the level of the oestrogen surge, the luteininsing hormone surge and the duration of the follicular phase when normal cycles were compared with those having a defective luteal phase.

Subnormal progesterone production by the corpus luteum will result in inadequate secretory change in the endometrium.

Hyperprolactinaemia may be a rare cause of inadequate progesterone production (29). Factors affecting hypothalamo-pituitary dysfunction may also cause a defective luteal phase eg extremes of weight, extremes of reproductive life, strenuous exercise and stress. However, in at least five studies investigating the level of progesterone in the luteal phase in

patients with delayed endometrial development, the percentage of patients with subnormal levels of progesterone never exceeded 50% ; in one of them it was 55%. (29,30,31,32,33). Investigation of the possibility that luteal phase defects may be due to an inadequate progesterone hormone-receptor binding has been investigated with conflicting results. Both decreased and increased concentrations of cytosolic and nuclear progesterone receptor have been reported. Problems with technique is probably the reason for these results (34).

Many methods have been used to evaluate the luteal phase, these include basal body temperature, endometrial biopsy, ultrasonographic measurement of the endometrial thickness and even measurement of endometrial proteins.
These methods seek to evaluate, not only, if the luteal phase has been produced but also if it is adequate.

The **basal body temperatue** is a crude way of assessing the adequacy of the luteal phase and relies on the thermophilic qualities of progesterone. Given a normal 28-day cycle, the presence of progesterone in the second half of the cycle will induce a rise in basal body temperature of circa 0.4 to 1.0 degree F.
Clinically it has found a use in assessing if ovulation has occurred. This was only used infrequently in the study group and usually serves to involve the mother in the investigation

process; something which has a positive psychological effect.

**Serum progesterone assays** is the standard method to check for luteal phase defects in the miscarriage clinic and are taken in the mid-luteal phase to indicate if the cycle is ovulatory or not (35,36). The World Health Organisation (1984) recommended that a level of 18 nmol\l should be used to indicate if ovulation has occurred.

The level used to asses if the luteal phase is adequate or not is, however, higher at 30 nmol/l.

Different authors have recommended different times as being the optimal time to assess the adequacy of a luteal phase. Li et al (28) suggest that the early part of the phase is the more important while Daya (37) concludes that the late luteal phase is more important.

However, the wide variation of results of serum progesterone assays (38) makes this assay difficult to interpret and use as a basis for treatment. Serial progesterone assays through the luteal phase would obviously be more accurate but are difficult to accept by the patient. Blood sampling would be best timed with the LH surge to provide a standard reference point (39).

**Endometrial biopsy** became the gold standard to asses the luteal phase following the seminal publication by Noyes, Heertig and Rock in 1950 (40). The appearance of vacuoles,

changes in the nucleus, the presence of intra-luminal secretory material, oedema, vascular changes and the presence of pre-decidua are all used in sequence to date an endometrial biopsy, and if the changes are adequate in respect of the timing following ovulation. A lag of two to three days is considered suggestive of LPD.

This is, however, an invasive investigation although it can be done on an out-patient basis, and it is often only in retrospect that one can be sure that the biopsy was actually taken on the correct day. Having to repeat because the biopsy was possibly done a day or two early or late will not be acceptable to the patient besides being at a considerable cost.

**Ultrasound assessment of the endometrial thickness** has been shown to correlate with endometrial dating (41,42). Unfortunately, ultrasound cannot differentiate endometrial thickness due to secretory change from that due to excessive oestrogen stimulation.

Luteal Phase defects are diagnosed more frequently in the following clinical situations:

1. Recurrent miscarriage
2. Hyperprolactinaemia
3. Treatment with clomiphene citrate
4. Strenuous athletic training
5. Women at the extremes of reproductive life.

6. Women resuming normal menstrual function following birth, miscarriage or discontinuation of the contraceptive pill.
7. Hyperandrogenic states
8. Unexplained infertility
9. Hypothyroidism
10. Hyperstimualtion of the ovary (26)

Luteal phase defect has been repeatedly proposed as a cause of recurrent pregnancy loss (39,43,44) and it is one of the conditions which must be excluded when investigating women sufferring from recurrent miscarriage.

The treatment for luteal phase defect should be clomiphene with progesterone support and possibly human chorionic gonadotrophin if the patient complains of oligomenorrhoea.
The outcome of clomiphene treatment in luteal phase defect was very positive with five babies being delivered from a group of six (87.3%).
The use of clomiphene was a significant factor contributing to a positive outcome in the analysis of the data.
This contrasts sharply with the outcome in PCOS (vide infra) where no babies were delivered from a group of five patients.
I believe this is due to the fact that the two conditions exert their influence on miscarriage rates because of different reasons and while a 'simple' elevation of luteal progesterone

levels works in LPD it does not do so in PCOS.

It is our practice at the Miscarriage Clinic to instruct patients who are on clomiphene to start their luteal phase support treatment as soon as a diagnosis of pregnancy is made and not when they are able to see a doctor. The first weeks are crucial in this condition especially as fresh knowledge on the role of progesterone as a modulator of immunological processes in pregnancy is becoming available.

# POLYCYSTIC OVARY SYNDROME

Polycystic ovary syndrome is probably the single most important clinical entity in reproductive endocrinology. That it is a single clinical entity is a subject of much discussion and its history has been characterized by much debate as to the best method of diagnosis, its manifestations and, of course, management.

The truth of the matter is that it is a condition of such diverse clinical features that to put such a heterogenous group of patients in one group may not seem reasonàble. And yet, all these patients have particular features in common of which, probably, ovarian hyperandrogenaemia is the most important.

The condition was first alluded to by Chereau in 1844 when he described sclerocystic changes in the human ovary (45).

In 1935 Stein and Leventhal published their classical work on polycystic ovaries and its association with amenorrhoea (46). Since then a number of publications have made polycystic ovary syndrome probably the most important condition in reproductive endocrinology.

The first criterion to be employed in the diagnosis of PCOS was a raised luteinising hormone level which was first reported in 1958 and this became clinically useful when radioimmunoassay was introduced.

The association of polycystic ovary syndrome with insulin

resistance was first reported in 1976 (47) and in 1980 (48) the typical ultrasound appearance of polycystic ovaries were first described in 1981 (49). These were refined by Adams et al in 1985 and the ultrasound diagnosis of PCOS became accepted as the standard way of making this diagnosis (50).

Of the important metabolic disturbances associated with PCOS ovarian hyperandrogenaemia is the most common. PCOS patients have a characteristic hyper-response of androstenedione secretion in response to gonadotrophin stimulation. Rosenfield proposes that this is due to a dysregulation of androgen secretion, probably due to hyperactivity of the enzyme, cytochrome P450c17. Rosenfield suggests that PCOS may arise form either LH excess or an escape from desensitization (downregulation to LH stimulation).

Hypersecretion of luteinising hormone has been implicated as a cause of recurrent miscarriage in patients with polycystic ovary syndrome (51).

The ways in which elevated LH levels may affect the outcome of a pregnancy have been postulated to be:

- Direct inhibition of oocyte maturation inhibitor causing premature resumption of meiosis and the production of physiologically-aged oocytes.

- Direct effect on endometrial function, possible via abnormal prostaglandin synthesis.

- Indirect effect on either the oocyte or endometrium via altered androgen production.

- Production of abnormal glycoforms of LH causing abnormal signal transduction.

The concept of physiologically aged oocytes (52) is appealing if PCOS exerts its effect on miscarriage through elevated LH levels. The endometrium contains LH receptors (53) and women with PCO have altered synthesis of endometrial prostaglandins (54) which are important in implantation.
Elevated androgen levels, are the consequence of hypersecretion of LH and are themselves associated with early pregnancy loss (55,56).
Possibly women with PCO and insulin resistance produce abnormal glycoforms of LH with reduced immunoactivity and potency leading to poor signal transduction at receptor level (57). This will result in defective, or absent, action of luteinising hormone on both ovulation and endometrial maturation.
The incidence of Polycystic ovary syndrome in recurrent miscarriage clinics has been variously reported to vary from over 80% (64) to 50% (58,59).
The incidence reported in the study group with recurrent msicarriage was only 11.1%, which is lower than the experience

of most other clinics. The reason for this could lie in the fact that this group of patients included a significant number who had suffered only two recurrent miscarriages. In fact in the group of patients with strictly three recurrent miscarriages there was an incidence of 21.2%. This is very comparable to that reported from the general population and does not indicate a strong association between PCOS and recurrent miscarriage.

The incidence of PCO in a normal population using ultrasound is around 22% (60,61,62), and miscarriage rates of 30-33% have been reported in women with PCO without a past history of miscarriage (63).

It is, however, interesting to note that the majority of women with recurrent miscarriage who are found to have polycystic ovaries on ultrasound will be noted to have regular ovulation (55,62).

Also, the different incidences of PCO reported will reflect different diagnostic criteria. Although ultrasound diagnosis is the most uniformly accepted way to diagnose PCO, others still insist on biochemical evidence in order to make the diagnosis. In our clinic we rely on a combination of ultrasound features and biochemical results to make the diagnosis. Possibly if only ultrasound appearance is employed the incidence would prove to be higher. Certainly using two complimentary sets of criteria is more stringent than only one and logically fewer cases will meet both sets rather than only one.

The outcome of patients with PCOS was a 66.6% success; 10 babies were reported from fifteen patients with this condition. Just under half these patients did not receive any specific treatment and 3 babies were recorded while another three failed to conceive, and there was one miscarriage from this group.

This condition is often found to be associated with both infertility and recurrent miscarriage in the same patient. A period of years during which a patient sustains a series of miscarriages may well be followed by infertility, probably due to contributing age-related effects.

# TREATMENT

Clomiphene citrate was used both in patients with PCOS and with luteal phase defects. In all cases were this was used progesterone supplementation in the luteal phase was employed. The progesterone employed is dydrogesterone because of it is devoid of any androgenic effect and therefore safe to use when the fetus turns out to be a female.

In the first year of the study this was sometimes combined with human chorionic gonadotrophin but this was not continued as evidence for this was not very good (except in cases with oligmenorrhoea; see below).

In six cases with LPD, five babies were delivered and there was one miscarriage; while in five patients with PCOS there were five miscarriages i.e. no successful outcomes.

The rationale of clomiphene in LPD is stimulation of the follicular phase will improve progesterone levels in the luteal phase and this resulted in an 83% (5/6) success rate.

Although clomiphene will stimulate ovulation and improve outcome in cases of infertility associated with PCOS it is not generally considered to have a major impact on miscarriage rates associated with PCOS.

This impression is fully consistent with the outcome of the five patients with PCOS who were treated with clomiphene and unfortunately all experienced another miscarriage.

The treatment of PCO in recurrent miscarriage should address

the theory that hypersecretion of LH is the putative factor contributing to an increased miscarriage rate in this condition (59,64,65). An initial trial using buserelin suppression against straightforward clomiphene citrate (66) showed initial promise for this condition.

This treatment option (down-regulation with GnRH agonists followed by ovulation induction with human menopausal gonadotrophin) was employed for three patients with PCOS and two babies were delivered.

An improved success with this protocol can be envisaged because the presumed putatively elevated luteinising hormone (LH) is treated in this way and this may be evidence in favour of this theory.

A more recent randomised trial which tried to address this issue has shown that pre-pregnancy suppression of LH does not, however, improve the live birth rate amongst ovulatory women with PCO who hypersecrete LH (67).

Oligomenorrhoea is a common feature of PCO.

It is interesting to note that a study on human chorionic gonadotrophin (considered in the section on luteal phase defect) showed it to be effective in patients with oligomenorrhoea (68). This group of patients may represent cases of polycystic ovary syndrome who have anovulatory cycles.

Clomiphene will continue to be used as a first option both in luteal phase defect and polycystic ovary syndrome because it

is cheap, safe and effective. This approach is further justified by the overall positive effect of clomiphene in the results.

The unsatisfactory results in PCOS does not make GnRH agonists an automatic choice as these are expensive and labour intensive when ovulation induction is monitored. There is furthermore an increased risk of hyperstimulation syndrome with this protocol in PCOS when compared to clomiphene citrate.

# A REVIEW OF THE TREATMENT OF LUTEAL PHASE DEFECTS IN RECURRENT MISCARRIAGE

## *Oestrogens*

The story of the administration of oestrogens to women to prevent miscarriage is a very unfortunate one.

Diethylstilboestrol was a popular drug with obstetricians on both sides of the Atlantic right through from the 1950's to the 70's. The potential value of DES to reduce the risk of miscarriage was proposed by a number of studies (69). These trials did not use any controls and there was no attempt at blinding either the patients or the investigators.

A meta-analysis (69) of studies carried out at about the same time where patients were subjected to a more rigorous methodology shows however, that no benefit is revealed from the use of DES on the risk of miscarriage, prematurity, stillbirth or neonatal death. In one study (70) the odds ratio actually suggested an increase in the risk of miscarriage in patients who took the drug.

In spite of these results DES was prescribed for another 30 years until Herbst and Scully (71,72) reported and subsequently confirmed the link between maternal stilboestrol administration and the development of a clear cell adenocarcinoma of the vagina in female children born to these moth-

ers.

Other side-effects noted in offspring of mothers exposed to DES in pregnancy are vaginal adenosis, circumferential cervical and vaginal ridges (73), reduced fertility (74), and an increased risk of premature birth, perinatal death, spontaneous miscarriage and ectopic pregnancy (75).

The experience of the DES story should serve as a lesson to all of us who prescribe in pregnancy. Enthusiasm for any particular form of treatment must meet the requirements of safety before it is advocated.

## *Human Chorionic Gonadotrophin*

Human chorionic gonadotrophin is prescribed to patients who have a history of recurrent miscarriage (76,77).

HCG has a structure very similar to LH and exhibits similar pharmacological functions. It can be used instead of LH to induce ovulation.

An association between recurrent miscarriage and follicular phase hormonal aberration has been postulated by several authors (66,78,79). Further research supported a specific association between recurrent spontaneous miscarriage and PCOS (64,79).

It was also shown that oligomenorrhoea was ten times more important than any other identifiable risk factor in predicting a failed pregnancy in women with recurrent spontaneous mis-

carriage (80). They did not, however, find any abnormality of luteinising hormone in women with recurrent pregnancy loss and oligomenorrhoea.

In a review for the Cochrane Database of Systematic Review (81) on the effectiveness of HCG administration during early pregnancy on the risk of miscarriage in women with a history of recurrent spontaneous miscarriage, and further to investigate the effect of HCG on the risk of miscarriage in women with recurrent miscarriage who also had oligmenorrhoea, suggests that HCG may have a beneficial effect on the risk of miscarriage for all women with a history of recurrent miscarriage. This conclusion may, however, be biased by two early studies (Svigos 1982 and Harrison 1985) were the methodology was not very strict. A multicentre placebo controlled trial of early pregnancy HCG supplementation failed to show any benefit in pregnancy outcome (82).

The beneficial effect of HCG in women with recurrent spontaneous miscarriage and oligomenorrhoea appears to be substantial (83) although the number of patients involved in this study was small.

## *Progestagens*

Progestagens have been widely prescribe in an effort to reduce the risks of miscarriage (84).

They are well known to be essential to the maintenance of an

early pregnancy as evidenced by falling progesterone levels in a failing pregnancy (85). They may exert their action either through inhibition of myometrial activity (86) or through suppression of prostaglandin synthesis (87,88).

Many studies have been undertaken in order to assess the efficacy of progesterone in order to avoid miscarriage. Unfortunately, the majority were faulty in their design.

Often therapy was commenced when a fetal heart was detectable, by which time all the hormonal events would be complete, the patients selected were not strictly chosen because of a diagnosis of luteal phase defect.

If hormonal supplementation is effective it should be given from the time of ovulation and continued throughout the critical stage of implantation up until nine weeks stage when the corpus luteum becomes less important (89).

Because of the controversy generated from what most observers considered to be inadequate trials a number of meta-analytical reviews have been undertaken.

In 1989 Goldstein et al (90) published a meta-analysis of ran-

domized controlled trials of progestational agents in pregnancy. Their inclusion criteria were:

1. All study patients must be considered to be in high risk pregnancies
2. The study report must state that patients were randomly or quasi-randomly allocated to the treatment or control study group
3. the study group patients received progesterone or progestogen
4. the paper must provide data on outcome of pregnancy including livebirths and miscarriage or neonatal death.

The meta-analysis was eventually based on 15 RCT's although over 140 reports were found in an electronic search. The result revealed an odds ratio for miscarriage vs liveborn babies of 0.97 (95% CI 0.65-1.44) suggesting that there is no benefit to the use of progestogens in this circumstance but not excluding the possibility of either benefit or detriment. As the authors comment in their discussion this reflects precisely the spectrum of opinion in medical textbooks with some authors advocating the use of progestogens, others considering its use inappropriate and others remaining equivocal about

its use.

Another meta-analysis was reported by Daya (91). This meta-analysis was based on three studies which were performed in 1953 and two in 1964. His inclusion criteria were:

1. Patients were allocated to treatment or control groups on a random or quasi-random basis.
2. Treatment with progesterone (or progestogen) was started in the first trimester.
3. The control group received placebo
4. Women recruited to the studies had three or more previous consecutive miscarriages.

His conclusion was similar to Goldstein's.

A third meta-analytical review was published in 1990, i.e. a year later by Kierse (92). He insisted that a distinction must be made between the different progestational agents. He concentrated his meta-analysis on placebo-controlled studies which involved the prophylactic use of a single progestagen i.e. 17 alpha-hydroxyprogesterone caproate. Four of the seven eligible studies provided data on miscarriage. His conclusion was that 'there is currently no evidence from controlled trials to justify the clinical use of any progestational agent to prevent miscarriage'.

Two reviews (93,94) which addressed the effects of progesterone administration on adverse pregnancy outcome (miscarriage, stillbirth, neonatal death and prematurity) and the prophylactic use of progesterone to prevent miscarriage and

preterm birth again concluded no demonstrable benefit in terms of risk of miscarriage.

The actual odds ratio (and confidence interval) of all these studies is compatible with either a beneficial or a detrimental effect.

The final word on the role of progestagens in recurrent miscarriage has obviously not yet been said. It is a drug which will continue to be prescribed for the foreseable future as our own results have shown it is a drug which is free of adverse affects and which continues to show its effect in observational studies. Of course, in the era of evidence based medicine one would like to base one's prescribing decisions on sound randomized controlled studies. Unfortunately, these are not forthcoming and the best that meta-analysis can achieve is that the drug may be beneficial, but may also be detrimental! Until more sound scientific evidence becomes available one is left to decide on the appropriateness of prescribing progestagens on the available evidence, experience, and as thorough an understanding of the processes of miscarriage as possible. It is my opinion that progestagens, especially, non-androgenic examples such as dydrogesterone (95,96) offer a pharmacological effect on the pregnant uterus which is beneficial. This probably due to relaxation of myometrial muscle and also to an effect on immuno-modulation at implantation (97) which may finally provide the link between endocrine and immunological factors affecting miscarriage.

The role of progesterone induced blocking factor in the

immunological process of pregnancy is discussed later.

## *Clomiphene citrate*

Clomiphene citrate has been proposed as a treatment option in LPD (98). It acts by blocking the negative feedback effect of oestrogen on the pituitary thereby increasing follicle stimulating hormone levels. As was discussed earlier this is likely to improve the luteal phase.

It has found considerable application in the treatment of infertility and in our own group proved valuable in the treatment of recurrent miscarriage.

The possible treatment options for luteal phase defect are therefore ;

Gonadotrophins

Clomiphene citrate

Progestagens

Human chorionic gonadotrophin

Combinations of the above

# CHAPTER III

The third group is the **autoimmune or antiphospholipid group** with 14 cases. There were 9 babies delivered from this group; a further 3 miscarriages and two cases where no further pregnancy was recorded.

The treatment of autoimmune causes of recurrent miscarriage offers a number of possibilities.
The options are no treatment, low-dose aspirin, low-dose aspirin and prednisolone and low-dose aspirin and heparin.
The low-dose aspirin and prednisolone arm option is at present no longer in use as will be discussed further, and the recommended treatment of confirmed anti-cardiolipin antibody positive patients is low-dose aspirin and heparin.

All treatment options had in fact quite a good outcome and it can be said that autoimmmune causes of recurrent miscarriage are amenable to treatment which makes it all the more important to exclude.
A differentiation into low-titre and high-titre disease is useful as low-titre cases may have a positive outcome if no treatment is given. Two such cases were recorded although it has to be said that in the first year of the clinic doubts about the safety

of low-dose aspirin and especially prednisolone in pregnancy contributed to the couple refusing any treatment.

It is unlikely that high-titre disease will result in a baby if no treatment is given especially in confirmed antiphospholipid antibody syndrome.

Our experience led us to propose warfarin in two such cases. The first patient was allergic to heparin and the second has suffered no less than 12 miscarriages in spite of heparin treatment in the last two, although by then she was over 40 years of age.

The former patient delivered two live healthy babies on warfarin with no further thrombo-embolic episodes (99). The safety of warfarin in pregnancy should be reassesed although it is very difficult to do so in view of the fact that it is now classified as an established teratogen and therefore any

prospective trial impossible to implement.

## IMMUNOLOGICAL ASPECTS OF RECURRENT MISCARRIAGE

A pregnancy is a successful allograft. It is clear that immunologically a pregnancy will only share the maternal antigenic contribution with her host while the paternal contribution will be foreign and therefore liable to rejection.

The implanting blastocyst will express these paternal histocompatibility and stage-specific antigens and at this time the endometrium and decidua will contain a number of leukocytes, including T lymphocytes and macrophages (100).

Bearing this in mind it is very tempting to hypothesise that a failure in the normal immune mechanisms that permit maternal acceptance of the fetal-placental semi-allograft to be a cause of miscarriage.

Four antibody-mediated (humoral) hypotheses have been proposed as a cause of recurrent miscarriage.

Of these, antiphospholipid antibodies are the only proven cause of recurrent miscarriage and will be dealth with in detail later.

Other proposed mechanisms involve antisperm antibodies, antitrophoblast antibodies, and blocking antibody deficiency.

Although antisperm antibodies were initially implicated as a cause of miscarriage (101) subsequent work did not uphold

this view (102) and they are not currently considered clinically important.

The hypotheses that antitrophoblast antibodies are involved has not been substantiated, while the concept of a maternal blocking antibody deficiency has received much attention after its initial proposal (103).

This theory was founded on three suppositions:

1. there is an antifetal, maternal cell-mediated immune response that develops in all pregnancies that must be blocked
2. blocking antibodies develop in all successful pregnancies that prevent this maternal, antifetal, cell-mediated immune response
3. in the absence of blocking antibodies, abortion of the fetus always occurs.

These suppositions did not stand to the test, however (104).

Mixed lymphocyte cultures reactivities between maternal responder and paternal stimulator cells were originally used to determine whether blocking activity in response to uncharacterized serum factors existed (103).

Later, however, it was shown that some women with successful pregnancies did not produce serum factors capable of mixed lymphocyte culture inhibition (105) or to produce antibodies against paternal human lymphocyte antigens (106).

The demonstration that agammaglobulinaemic women may

reproduce successfully makes any hypotheses regarding the necessity of a blocking antibody in order to achieve a successful pregnancy untenable (107).

A further four mechanisms have been proposed as causes of recurrent miscarriage involving the cellular immune system.

1. TH1 cellular immune response to reproductive antigens
2. TH2 cytokine, growth factor and oncogene deficiency
3. Suppressor cell and factor deficiency
4. Major histocompatibility antigen expression

TH1 And Th2 cells represent two polarized forms of T helper cells, and as the major functional subsets of Th cells they mobilize different types of effector responses (108).
Th 1 cells induce several cell-mediated cytotoxic and inflammatory reactions via interleukin-2, interferon-gamma, and tumour necrosis factor-beta, while Th2 cells are associated primarily with the provision of help for B cell antibody production via the Th2-type cytokines, IL-4, IL-5, IL-6, and IL-10 (109).
It has been proposed that Th1-type cytokines are deleterious to pregnancy (110) while Th2-type immune responses have been proposed to represent the normal responses in a successful pregnancy (111).
It is therefore hypothesized that the conceptus may be a target

of local, cell-mediated, immune responses resulting in abortion. In these patients induction of an immune response because of trophoblast, sperm, microbial or other antigens activate a cellular immune response mediated by TH1 cytokines, interferon-gamma and tumour necrosis factor which have been shown to inhibit in vitro growth of embryo and trophoblastic tissues. Studies involving, in all over 2000 women, have shown that 50% of women with idiopathic recurrent miscarriage will have evidence of an abnormal TH1 cellular immune response. These are found in less that 3% of women with normal reproductive histories (112,113).

It may also be proposed that the association between mycoplasma and chlamydial infections and recurrent miscarriage (114) may be due to activation of TH1 immune respons-

es (115).

## The Differences between T helper 1 and T helper 2 cells :

| | Th 1 | Th 2 |
|---|---|---|
| Immunological Response | strong cell-mediated<br>weak humoral | strong humoral resp<br>weak cell-mediated |
| Cytokines Produced | Interferon-1-gamma<br>Interleukin 2, 9 &13<br>Tumour necrosis factor | Interleukin 4, 5, 6, 10. |

The effects of cytokines are inhibition of trophoblast out-growth, apoptosis of trophoblast cells and therefore miscarriage.

In essence the evidence suggests the theory that a healthy ongoing pregnancy is a Th2 process while a miscarriage is the result of a Th1 response.

The balance of these two systems is modified by the presence of a mediator protein termed Progesterone Induced Blocking Factor (PIBF). Normal pregnancy is characterized by the presence of progesterone receptors in peripheral blood, and the ratio of progesterone-receptor positive cells increases as pregnancy progresses. However, the ratio of these progesterone receptor-positive cells is significantly lower in women who are at risk of miscarriage. People who have received transplants or blood transfusions have a similar ratio of progesterone-receptor-positive cells as pregnant women. This suggests that alloantigenic stimulation, represented by the fetus in pregnant women is responsible for the increase in lymphocyte progesterone receptors. PIBF favours the production of Th2 cytokines with very few Th1 cytokines being produced resulting in a low natural killer cell activity and normal pregnancy outcome. In contrast, if no PIBF is available, the concentration of Th1 cytokines increases resulting in high NK cell activity and miscarriage. These facts have been widely accepted for animal models. Should they be true for humans they would offer an exciting new explanation for the observed protective effect of progesterone on the maintenance of a pregnancy.

A recent application of this is the estimation of NK cell activity and treating with prednisolone if this is found to be elevated.

Other immunologic cytokines, growth factors and oncogenes

have been identified in human pregnancies and a deficiency of these has been theorized to lead to miscarriage (116).

Suppressor cell and factor deficiency is another cellular immune mechanism proposed to be a cause of recurrent miscarriage. Most of this work has, however, been done on mice. Evidence of suppressor cell activity in endometrial biopsies of patients with failing pregnancies following IVF\ET has been reported (117). Deficiencies of suppressor cell/factors may represent changes consequent on miscarriage rather that being causal in nature.

The human syncytiotrophoblast does not normally express the major histocompatibility complex (MHC) class I or II antigens (118). Trophoblast cannot therefore be identified and targeted by MHC-directed cytotoxic T cells and it is also resistant to natural killer cells. Interestingly, interferon-gamma has been shown to induce the expression of MHC antigens on trophoblast thereby making it liable to the above-mentioned cytotoxic T cells (119). Although attractive this theory was not supported by a histochemical study of placentas from spontaneous miscarriages (120).

Lastly the effect of a deficiency of complement regulatory proteins has not found any support form studies on trophoblast from elective, spontaneous and recurrent abortions. In these studies trophoblast was found to express complement regulatory and binding proteins (120).

# THE ANTIPHOSPHOLIPID ANTIBODY SYNDROME

The antiphospholipid syndrome was first described by Hughes in 1983 (121), is now recognized as an important prothrombotic condition associated with the presence of a number of autoantibodies. The association of antiphospholipids with recurrent fetal loss was described between 1985 and 1987 (122,123).

Antiphospholipid antibodies were first detected by Wassermann in 1906 and first described in patients with systemic lupus erythematosus (SLE) by Conley & Hartmann in 1952. They were initially considered to be part of a connective tissue disorder but have since come to be considered as being part of an independent syndrome.

**Phospholipid biochemistry**. Phospholipids are constituents of all cell membranes. They consist of a glycerol backbone, two esterified fatty acid chains (one saturated and one unsaturated) and a phsophodiester-linked alcohol side chain. Phospholipids exist within the cell membranes in at least two phases : most commonly in a lamellar bilayer in which the hydrophobic fatty acids of the inner and outer leaflets are directly apposed whereas the hydrophilic, negatively charged (anionic) alcohol groups of the inner medium are exposed to the cytoplasm and the hydrophilic neutral alcohol groups of

the outer leaflet are exposed to the extracellulatr fluid and 2) a rarer, cone shaped, transient, hexagonal phase induced by excess calcium, low Ph and/or excess phosphatidylethanolamine (PE) constituents (124). There are both exogenous and endogenous stimuli for APA production. The former include APA that arise as a consequence of infection and are not associated with thrombosis while the latter are associated with endothelial membrane perturbation and are often associated with thrombosis (eg lupus anticoagulant and anticardiolipin antibody). The first aPl associated with infection were discovered in 1941 in response to Treponema pallidum (125). Other viral, bacterial and parasitic infections including measles, mumps, varicella, parvovirus, adenovirus, Ebstein-Barr virus, pneumoccal pneumonia, mycoplasma, Lyme disease and malaria can induce transient aPL expression. Johnstone et al (126) observed a 24 % prevalence of aPL among HIV infected pregnant patients without an increase in adverse pregnancy outcomes. Those aPL that arise from endogenous stimuli generally recognize protein-phospholipid complexes. They are thought to be generated in reponse to aberrant immunoregulation accompanying autoimmune disorders. More recently, however, it has been theorized that these aPL can be generated by any pathological condition which causes anionic phospholipids, normally resident on the inner cell memebrane, to be expressed on external membrane leaflets of endothelial cells or induce hexagonal

phase phospholipids. This, in turn, permits a number of phospholipid-binding proteins to be presented to the immune system in unique neoantigenic conformations giving rise to aPL. Such a mechanism would account for the appearance of aPL in a wide variety of conditions each of which is associated with disturbances in endothelial homeostasis (eg autoimmune disorders, thrombosis, Sneddon's Syndrome, atherosclerotic vascular disease and pregnancy). Two classes of aPL resulting from endogenous stimuli include; those that prolong phospholipid-dependent in vitro clotting assays, LAC and those recognized by immunoassays. Evidence that LAC and ACA are unique include : the frequent discordancy between LAC activity and ACA reactivity among patients with either aPL (127); the absence of a correlation between ACA concentrations and LAC activity; the finding that plasma fractions containing LAC activity can be separated from those with ACA reactivity using polyacrylamide-phospholipid affinity column chromatography; and the unique phospholipid-binding protein co-factors recognised by the two classes of prothrombotic APA (128).

**APA prolonging phospholipids-dependent clotting assays: LAC.** This class of aPL was first described by Conley and Hartmen in 1952 (129). They reported two patients with SLE who displayed biological false-positive serological tests for syphilis and a plasma inhibitor of in vitro clotting assays. This inhibitor was subsequently termed the lupus anticoagu-

lant (130), although this term is misleading since many patients with these antibodies do not have lupus and its primary clinical sequelae are thrombotic events. Circulating LAC's that lack aPL reactivity react to the complex of anionic phospholipids and prothrombin (128).

Their in vitro anticoagulant activity can be neutralized by the addition of hexagonal but not bilayer phase phospholipids (131). Thus the presence of hexagonal phase PE-enriched phospholipids may induce an unusual prothrombin conformation rendering it antigenic. There is also evidence that hexagonal phase phospholipids are inherently immunogenic and that occasional LAC and APA recognize cryptic phospholipids epitopes not complexed with proteins (132).

**APA identified by ELISA : ACA.** In 1983 Harris and associates (133) demonstrated that there was a class of APA present in patients with autoimmune diseases which reacted against anionic but not neutral phospholipids present on the solid phase of an immunoassay system. These initial APA immunoassays were intended to be quantitative VDRL assays and, therefore, used cardiolipin as the target substrate giving rise to the term anti-cardiolipin antibodies. However, it seems that ACA are polyspecific, recognising all anionic phospholipids (134). As noted above it has been demonstrated that ACA do not recognise anionic phospholipids alone but rather react with a protein-anionic phospholipids complex (134). Highly purified ACA, free of other serum proteins, do

not bind to purified anionic phospholipids in immunoassays . In contrast these highly purified ACA do bind to a heavily glycosylated 57kD anionic phospholipids-binding protein termed beta-2 glycoprotein-1 (apolipoprotein H). Indeed beta2GPI is recognized by these antibodies when bound to anionic phospholipids but not when bound to nitrocellulose, heparin or affigel (135). It has been estimated that one third of APA with anticoagulant activity react to beta2GPI-phospholipid bilayer complexes whereas two-thirds recognize prothrombin-hexagonal phase phospholipids complexes (136)

**The Primary Antiphospholipid Syndrome (APS)** as described by Harris in 1987 has the following features :

Clinical features

- Recurrent venous or arterial thrombosis
- Recurrent fetal loss
- Thrombocytopenia

Serological features

- The presence of the lupus anticoagulant
- The presence of anticardiolipin antibodies IgG or IgM type in a titre in excess of 20 G/MPL.

Patients with APS can present to a variety of clinical specialists, obstetricians, haematologists, neurologists, cardiologists, rheumatologists and dermatologists. It is also essential to appreciate that aPl are not specific to APS. Transient anti-

body titres may be triggered by intercurrent infection, use of drugs and persistent aPL positivity may be a feature of chronic infection eg syphylis, hepatitis, and HIV. In these cases patients are not prone to the thrombotic consequences of APS and may be neither beta-2-glycoprotien-1 or prothrombin dependent. Some of the drugs that have been associated with aPl titres are; chlorpromazine, hydralazine, propanolol, procainamide, quinidine, dilantin, valproic acid, amoxicillin and streptomycin (134).

In 1999 the clinical and laboratory criteria were reviewed in The International Antiphospholipid Symposium in Sapporo, Japan and three different types of pregnancy loss were determined as a clinical criterion for APS ;

1. One or more unexplained deaths of a morphologically-normal fetus at or beyond the tenth week of gestation with normal fetal morphology documented by ultrasound or direct examination of the fetus.
2. One or more premature births of a morphologically-normal neonate at or before the thirty-fourth week of gestation because of severe pre-eclampsia or placental insufficiency.
3. Three or more consecutive spontaneous abortions before the tenth week of gestation with maternal anatomic, hormonal abnormalities, and paternal and maternal chromosomal causes excluded (137).

Other clinical manifestations have been described, namely chorea, heart valve disease (verrucous endocardial lesions) ,livedo reticularis and thrombotic events leading to stroke, spinal thrombosis, pulmonary embolism and Budd-Chiari syndrome. It also became clear with time that the presence of antiphospholipid antibodies was not only associated with recurrent fetal loss but also with other adverse obstetric outcomes. An association between aPL and intra-uterine growth retardation was described in 1991(138). This was confirmed by others and more recently by Birdsall et al in a study on the presence of aPl and IVF (139). Other complications are hypertension and pre-eclampsia. The risks for perinatal morbidity increase with increasing amounts of phospholipid (140). An increased perinatal risk due to antiphospholipids was first reported in 1985 (141), this increased risk of complications may represent a continuum of adverse pregnancy outcome starting not with miscarriage but with infertility indicating an immunological cause for infertility (142).

**Laboratory Testing for APS**

The diagnosis of APS relies on the demonstration of the presence of either LA by coagulation tests or aPL by solid phase immunoassay. The latter typically employ cardiolipin as antigen. At present, these traditional tests remain the mainstay of laboratory investigation of APS, and it is clear that both LA and solid phase type assays must be employed for the detec-

tion of aPL. Reliance on just one type of assay may lead to false negative results. There are detailed guidelines for the performance of assays for aPA (143) (see methods).
The following points will have to be considered when interpreting tests for antiphospholipid antibodies :

1. a firm diagnosis of APS requires a clear clinical history and persistently positive test results for aPL
2. LA tests are indirect, not entirely specific and of variable sensitivity
3. more than one type of LA test will need to be done to make the diagnosis accurately
4. anticoagulant therapy will prolong clotting times and interfere with the detection of LA
5. anticardiolipin antiboby tests are sensitive but transient positive test results are common
6. both LA tests and aCL (IgG and IgM) assays are required to diagnose or exclude APS
7. in order to avoid misinterpretation of test results requires knowledge of their accuracy, reproducibility, sensitivity and specificity. (144).

The prevalence of aPL in women with recurrent miscarriage has been reported to be 7-42%. This variation is surprising but is due to a lack of standardisation of laboratory protocols used to detect aPL and to the inclusion of patients with transiently positive test results. Using a comprehensive method-

ological approach with testing for both aCL and LA, the prevalence of a persitently positive result is about 15% (145). Our own incidence of antiphospholipid antibody positive cases was 10.3% which compares well with this result . The size of the problem in Malta is therefore similar to what is expected from the experience of other clinics.

It is important to appreciate that in women with recurrent miscarriage due to antiphospholipid antibodies the prospective fetal loss rate has been reported to be as high as 90%

(145).

## THE PATHOPHYSIOLOGY OF APS

The pathophysiology of the antiphospholipid syndrome is uncertain. Most of the attention has focused on the pro-thrombotic nature of the condition leading to thrombosis of the utero-placental circulation.

This theory has led to extensive studies on the placental bed and the descriptions have ranged from normal placentas to small infracted ones and those with extensive atherosis (146). In a study on ten patients with SLE Abramowsky et al (146) reported that the predominant lesion in half the patients was a necrotizing decidual vasculopathy, associated in a few arterioles with an inflammatory component and in most with fibrinoid necrosis and an atherosis pattern. These histologic lesions, particularly the latter, are identical to those described in eclampsia. The consequent decidual vascular injury results in variable placental ischaemia, which leads to impaired placental function and fetal death. De Wolff in 1982 (147) described very similar pathological changes in patients with lupus anticoagulant. Immunoflourescence studies have shown the deposits in the vessel walls to be immune complexes and these have been replicated in animal models (mice) by Branch et al (148). It is unclear whether decidual vasculopathy is the primary event or whether thrombosis in the decidual vessels or intervillous spaces causes these changes in the vessel walls.

More recent histological work however, has disputed the thrombotic mechanism of fetal death in APS. Out et al (149), in a survey of 17 placentae from women with aPL found no evidence of thrombosis in 18% of cases and this was confirmed in a study on placentae in pregnancies which ended before sixteen weeks gestation (150). While thrombosis is a plausible cause of fetal death in second trimester miscarriages it is unlikely to be at fault in early recurrent miscarriage. Either another mechanism is at fault in early miscarriages or a dual mode of action is present.

Antiphospholipid antibodies may exert their thrombotic effect either through an effect on platelets or on the endothelial cells. It has been demonstrated that both lupus anticoagulant and anticardiolipin antibodies require plasma protein cofactors to exert their action. In the case of LA, prothrombin is the cofactor (151) while for ACA it is beta-2-glycoprotein-1 (152). It has also become clear that antiphospholipid antibodies do not bind directly to negatively charged phospholipids but to either the protein/phospholipids complex or to the pro-

tein modified to the phospholipids' surface (153).

**Possible modes of action of antiphospholipid antibodies:**

## *Effect on platelet function*

Activation of platelets leading to a release of the procoagulant thromboxane. Since the negatively charged phospholipids to which APA bind are on the inside of the platelet membrane, the platelets must have been activated prior to the APA can bind to them.

It has been suggested that beta-2-glycoprotein-1 can bind to platelets and serve as an epitope for APA binding, leading to platelet aggregation and subsequent thrombosis. Beta2GP1 is a natural inhibitor of the intrinsic coagulation pathway (contact activation), of platelet prothrombinase activity and ADP-induced platelet aggregation.

## *Effect on endothelial cells*

**Inhibition of prostacyclin production by vascular endothelial cells.**

Prostacyclin, a potent vasodilator, is produced by endothelial cells via a pathway which requires the release of arachidonic acid from the phospholipids of the cell membrane by phsopholipase A2. It has been proposed that aPL reacts with

the phospholipids in cell membranes and inhibit the production of prostacyclin by the vascular endothelium, thereby promoting thrombosis (154). In this experiment it was shown that the serum of patients with LA could inhibit the production of prostacyclin and this was reversed by the addition of free arachidonic acid, suggesting that the LA was inhibiting the release of arachidonic acid from membrane phospholipids. This work was confirmed by some (155), but not all (156).

**Inhibition of the protein C/S anticoagulant system.**

Protein C acts as an anticoagulant by inhibiting the activated coagulation factors Va, VIIIa and the platelet bound Va-Xa complex (157). Two steps in this pathway are phospholipid dependent and may be inhibited by aPL:

1. Protein C is activated by thrombomodulin – a protein present on the surface of vascular endothelial cells. Thrombomodulin must be bound to phospholipids to yield optimum protein C activation and aPL can inhibit the in-vitro activation of protein C by thrombomodulin (158).

2. Once activated, protein C requires a cofactor, designated protein S, which facilitates the bonding of activated protein C to the platelet (159). Once bound to the platelet membrane, the protein C\protein S complex then inhibits coagulation factors Va and Xa. Several groups have demon-

strated that aPL can interact with phospholipids and inhibit the protein S-dependent anticoagulant activity of activated protein C. This mechaniasm has, however, not been consistently reproduced and it may be true in only a limited group of patients with aPL.

## *Inhibition of antithrombin III activity*

Endothelial cells express on their surface heparin-like molecules-glycosaminoglycans (GAGs) which activate antithrombin III (160). The GAG heparin sulphate is believed to be the physiological activator of ATIII and is particularly important as an anticoagulant in the microcirculation. It has been shown that 11% of patients with aPL have antibodies which cross-react with heparin and can inhibit the activation of ATIII (161). Therefore inhibition of GAG-dependent ATIII activity may also be a mechanism of action for some aPL.

## *Interference with the placental anticoagulant protein Annexin V.*

Human annexin V, also termed placental anticoagulant protein I and endonexin II, is a protein which like beta2GPI also binds to exteriorized anionic phospholipids to render their surfaces nonthrombogenic (162). Annexin V has been immuonlocalised in the placenta to syncytiotrophoblast

microvilli and has been shown to be constituitively expressed by cultured trophoblasts derived from term placentae (163). At this site it is in a position to impede intervillous thrombosis and promote uteroplacental blood flow. It has been reported that immunostaining for annexin V is diminished on the placental villous syncytiotrophoblasts of patients with high concentrations of APA and a history of recurrent stillbirths or late pregnancy losses (164). Because annexin V inhibits APA binding to beta2GPI phospholipids complexes (165), it may be that APA may conversely, cause displacement of annexin V from the syncytiotrophoblast surface and so render it procoagulant. It is also possible that APA-mediated interference with this annexin V may also disrupt villous trophoblast synctializatiuon providing an alternative mechanism for abnormal placental function, and possible miscarriage.
Any form of endothelial cell damage may lead to increased procoagulant activity or impaired fibrinolytic responses (166).

No single pathogenetic mechanism of action for aPL has been identified, but a number of different mechanisms may operate, each of which can explain the activity of some cases of aPL. Keeping in mind that antiphospholipid antibodies are such a diverse group of antibodies, it is not surprising that different mechanisms may operate for different cases.

Branch et al from Utah have argued that there are two distinct groups of patients with anitphospholipid antibodies with different clinical histories. In their experience over 85% of patients with APS have a history of at least one fetal death. In a retrospective analysis of 366 patients from their miscarriage unit (167) 76 of which were positive for antiphospholipid antibodies. Both groups were similar for rates of previous pregnancy loss but 50% of the prior losses in women with antiphospholipid antibodies were fetal deaths compared to less that 15% in those without. The specificity of fetal death for the presence of antiphospholipid antibodies was 76% compared to only 6% for two or more pre-embryonic or embryonic losses without fetal death. They concluded that fetal death, not pre-embryonic or embryonic loss is most specific for antiphospholipid-related pregnancy loss.

The significance of low positive IgG or IgM anticardiolipin antibodies is questionable (168). Silver et al in this study reported that women with low positive IgG anticardiolipin or isolated IgM anticardiolipin had no greater risk for anticardiolipin related events as women who tested negative. Also these women did not have the same risk for anticardiolipin related events. Furthermore patients with low-level IgG anticardiolipin antibodies did not have the same clinical background typical of patients with APS (169).

The titre of antibodies correlates with the risk of pregnancy

loss (most often fetal) and with the clinical risks the mother is exposed to, namely the risk of thrombosis. This is also exemplified by the fact that not all women with antiphospholipid antibodies and recurrent pregnancy loss require anticoagulation therapy for improved pregnancy outcome (170). On the basis of this evidence it has been postulated that two different patient populations positive for antiphospholipid antibodies exist:

1. Definite (or classic) antiphospholipid syndrome: those with lupus anticoagulant or medium-to-high levels of IgG anti-cardiolipin antibodies and a fetal death, thrombosis, or neonatal death after delivery for severe pre-eclampsia or fetal distress.
2. Recurrent pre-embryonic or embryonic pregnancy loss and antiphospholipid antibodies.

# THE TREATMENT OF APS

The immunological nature of the condition has led to the application of all known agents which have immuno-suppressive properties to treat the condition, with diverse results.

## *Prednisone*

The first report of the successful treatment of patients with antiphospholipid antibodies was in 1983 by Lubbe et al (171). This was a study on six pregnant women whose previous 14 pregnancies ended in miscarriage. A diagnosis of systemic lupus erythematosus was made in four of the patients while antinuclear antibody was demonstrated in all six. All patients had prolonged activated partial prothrombin times and kaolin clotting times which could not be corrected by dilution of test samples with normal plasma. In view of the characteristics of the patients immunosuppressive doses of prednisone were employed i.e. 40-60 mg daily together with aspirin 75 mg daily. Suppression of the lupus anticoagulant was achieved in five patients all of whom gave birth to live infants. APTT values had returned to the normal range after 9-16 weeks of treatment in the five women who had a successful outcome. In the sixth patient an intrauterine death at 16 weeks was reported and the APTT was only partly corrected.

In their conclusions the authors recommended the screening of patients with histories suggestive of the presence of lupus anticoagulant as prednisone in combination with aspirin offered hope of a successful pregnancy.
This experience was shared by Branch W et al (122) who treated eight women with prior fetal death and lupus anticoagulant with prednisone 50 mg daily and aspirin and five delivered live children. Complications, including intra-uterine growth retardation were noted but these may have been due to the disease process rather than the treatment.

In 1988 Loskshin et al (172) published a paper eloquently entitled 'prednisone does not prevent recurrent fetal death in women with aPL'. His conclusion is drawn from a prospective study of over 100 pregnancies complicated by systemic lupus erythematosus and antiphospholipid antibody. They evaluated 30 pregnancies in 25 **asymptomatic** women all having a 'high-titre' of antiphospholipid antibody, i.e. in other words these patients could not fulfill the criteria for SLE according to the American Rheumatism Association (173). Strictly speaking therefore the two groups of patients treated by Lubbe were not the same as those reported on by Lockshin. In his therapy study Lockshin used four treatment arms; a. no treatment, b. aspirin 80 mg daily, c. aspirin plus prednisone 30 mg daily for at least four weeks, then continued or tapered to a lower dose when toxicity was evident, but maintained at

least 10 mg daily and d. prednisone 60 mg daily for four weeks then continued at reduced doses until delivery.

Fetal outcome was analysed as a function of Maternal race, the presence or absence of SLE, IgG and IgM antiphospholipid isotype, presence or absence of lupus anticoagulant, other autoantibodies and fetal sex. Maternal age, lupus activity score, number of prior living children, number of prior fetal deaths and maximum antibody titres and lowest C3 and C4 complement levels were analysed as continuous variables. Risk for current fetal death was most apparent at antiphospholipid antibody levels >40 GPL units. Any prior fetal loss, regardless of cause, more than doubled the risk of future fetal death at all GPL levels.

The results of this study were not enthusiastic; 14 of 21 (67%) pregnancies of asymptomatic women were not successful. For women with a high antiphospholipid antibody titre but no prior fetal death, 3 of 9 (33%) pregnancies of asymptomatic women ended in fetal death. In neither the group with nor the group without prior fetal loss did aspirin improve prognosis; in fact, in the group with prior fetal loss, prednisone worsened the prognosis. One point which the authors discussed as an explanation for their results was the dosage schedule. In fact, no dosage studies have been published. They found that 60 mg prednisone daily too toxic to sustain throughout pregnancy and used 30 mg as a moderate dose and 40 mg as the full dose. The strength of their 21 patients together with the thoroughness of their study gave the authors conviction that their

conclusion was the correct one.

Further problems with the use of prednisone came with reports of complications to their use. Cowchock et al (174) reported that women treated with prednisolone plus aspirin had a significantly increased number of preterm deliveries, often associated with preterm rupture of membranes or the onset of severe pre-eclampsia when compared to those treated with heparin and aspirin. This was the same experience of Silver et al (175) who reported a significant excess of preterm delivery in women treated with prednisone plus aspirin as opposed to aspirin alone and concluded that prednisone treatment was an independent risk factor for preterm delivery.

In a more recent report Laskin et al (176) screened 773 non-pregnant women with at least two recurrent miscarriages for anticardiolipin antibodies and lupus anticoagulant. 385 patients were positive for at least one autoantibody and 202 of these became pregnant and they were randomly assigned to receive either prednisone and aspirin or placebo. The results showed that 65% of patients in the treatment group and 56% of those in the placebo group had live babies. More babies were however, born prematurely in the treatment group than in the placebo group. There was also a higher incidence of hypertension and diabetes in the treatment group than in the placebo group.

They concluded that treating autoantibodies and recurrent fetal loss with prednisone and aspirin is not effective in pro-

moting live birth and it increases the risk of prematurity. Considering this obstetric complication and the risk of development of hypertension, Cushingoid features, osteonecrosis, and pre-eclampsia, the use of immuno-suppresive doses of prednisone is questionable.

At this time, however, there is a renewed interest in the possibilities of prednisone as more knowledge becomes available on the T-helper modulatory effects on the immunological response to pregnancy. An increased level of N-K cells, indicative of a T-hlper I response is being investigated as an indication for immuno-suppression by prednisone.

## *Low-Dose Aspirin*

Peaceman et al and Carreras et al (177, 178) have demonstrated that immunoglobulin G fractions from patients with LA consistently alters platelet thromboxane production without altering prostacyclin production. Increases in production of the procoagulant thromboxane may therefore be a cause of fetal demise in APA-positive women. The mechanism of demise is presumably impaired blood flow in the utero-placental circulation, associated with micro-thrombosis and infarction of the placental bed (147). In a study on anticardiolipin positive patients treated with aspirin and flucortolone 10 mg daily it was shown that uterine blood flow (umbilical artery resistance index) improved in those patients who were

treated when compared to those who were not (179). Carroll also showed that Doppler flow velocity may be decreased in patients with lupus anti-coagulant (180). Though attractive this has not been universally accepted.

Low-dose aspirin has been shown to selectively inhibit platelet thromboxane production without affecting prostacyclin production (181). This action should improve blood flow in APA positive patients and improve the outcome for the fetus.

Aspirin was included in the original treatment protocols of antiphospholipid antibodies (Lubbe et al 1983 employed aspirin 80 mg and predniosolone 40 to 60 mg daily). Lockshin (172) used a combination of prednisone and low-dose aspirin, in a study that was mainly intended to address the value of steroids in this condition. In a study on 37 patients with antiphospholipid antibodies, including 15 with SLE, the most significant improvement was noted in the group who received low-dose aspirin in association with immunosuppression (182). Already at that stage it was noted that though a combination of prednisone and aspirin was to be supported, preliminary success had been reported with heparin and aspirin.

Following the interest in anti-coagulation as an approach to treatment rather than immunosuppression (183), Cowchock S F et al (174) conducted a study comparing prednisone and low-dose heparin. In this study aspirin 80 mg was prescribed

to both groups of patients. No attempt was made in this study to compare the groups to aspirin or placebo as the patients were considered to be at high risk of fetal death or thrombosis. This, however, indicates the degree to which low-dose aspirin became almost immediately standard treatment for APA positive patients.

Silver et al reported on a trial comparing prednisone plus aspirin vs aspirin alone (175). The results confirmed that treatment improved outcome but suggested that aspirin alone may be equivalent to combined therapy in achieving live birth. One explanation put forward by the authors for this was that the patient cohort manifested a less-severe form of antiphospholipid antibody syndrome in comparison to those reported by other investigators. This may have been due to inclusion of patients with low-positive antibody levels.

In their paper comparing aspirin and aspirin plus heparin Rai et al (145) comment on the variety of treatment regimens that have been proposed for this condition, i.e. corticosteroids, low dose aspirin, heparin, and immunoglobulins either as single agents or in combination. The problem that studies have included only small numbers of patients, varying entry criteria and treatment protocols and lack of standardization of lab-

oratory assays used to detect antiphospholipid antibodies.

## *Heparin*

Heparin is a mixture of polysaccharides with an average molecular mass of 15000 Da. On administration it stimulates the formation of irreversible complexes between antithrombin III and the activated serine protease coagulation factors (thrombin, XIIa, XIa, Xa, IXa, and VIIa). This results in an immediate anti-caogulant effect.

In 1982 Moe (184) administered 12500 units of heparin twice a day to a group of patients who had a previous history of perinatal death associated with placental infarctions and intra-uterine growth retardation. This treatment resulted in 15 live births.

The rationale for the use of heparin was the observation of infarction in placentas of affected pregnancies and reports that lupus anticoagulant may interfere with the activation of protein C by thrombin bound to thrombomodulin (185).

The first report of the use of heparin in anti-phospholipid antibody positive pregnancies was in 1990 (186). In this series fourteen of the fifteen pregnancies treated resulted in live births at a mean gestational age of over 36 weeks. One woman miscarried at 12 weeks after 6 weeks of treatment. They also reported that the incidence of placental infarction was reduced and mean birth weights increased. Successful

pregnancy outcomes were also reported by Gardlund B (187) and Parke (188).

Cowchock in 1992 (174) compared twice daily heparin to a prednisolone and aspirin combination. For both heparin- and prednisone-treated women the live-birth rate was 70% +/- 25%. However, significantly higher rates of pretem delivery associated with premature rupture of the membranes and serious maternal morbidity (usually pre-eclampsia) were observed for randomised women treated with prednisone.

In 1996, Kutteh, (189) published his study comparing fifty patients randomly assigned to receive either aspirin alone or aspirin and heparin in combination. The combination arm reported an 80% live birth rate compared to 44% for the aspirin only group. Besides the improved outcome, the tolerability of the treatment and the reduction in maternal complications was emphasised. The author suggests that some of the adverse obstetric outcomes that have been attributed to APAs may be exacerbated by treatment with prednisolone.

A year later Rai R et al (145) reported their study on 90 women with a history of recurrent miscarriage (median 4) and persistently positive results for antiphospholipid antibodies. Treatment was again randomly allocated to aspirin alone and heparin and aspirin in combination. Treatment with low dose aspirin in combination with heparin led to a significantly higher rate of live births (71%) than that achieved with low dose aspirin alone (42%).

These two studies conclusively showed that treatment with aspirin and low dose heparin leads to a significantly higher rate of live births than that achieved with aspirin alone in pregnant women with a history of recurrent miscarriage associated with antiphospholipid antibodies.

In the St Mary study most miscarriages occurred before 13 weeks gestation and the authors hypothesise that the combination treatment may promote successful embryonic implantation in the early stages of pregnancy and protect against thrombosis of the uteroplacental vasculature after successful placentation.

The use of heparin is associated with a number of potential complications including bleeding and immune thrombocytopenia. Osteopenia is another complication which is both dose-dependent and on the duration of its use. The decrease in bone mass is reversible on stopping treatment.

Low-molecular weight heparins, which have become available in recent years, like unfractionated heparin, also do not cross the placenta. Their use in pregnancy has been reported (190) and are considered safe to use. The most widely used to date is enoxaparin. The studies performed to date showed that LMWH are rarely associated with maternal adverse effects such as bleeding, thrombocytopenia or osteoporosis. They may be associated with a lesser degree of osteopenia than

unfractionated heparin (191).

## *Other Treatments*

Apart from steroids, aspirin and heparin, other agents have been employed in antiophospholipid antibody positive patients; such as dipyridamole (192), warfarin (99,192), immunoglobulin (193,194), plasmapharesis (195) and aza-

thioprine (196).

# CHAPTER IV

# PATHOLOGY OF THE UTERUS AS A CAUSE OF RECURRENT MISCARRIAGE

There were seventeen cases of recurrent miscarriage in this group of patients which were due to anatomical uterine pathology.

Seven of these were due to **cervical incompetence** while ten were due to **congenital uterine malformations.**

As will be discussed the diagnosis of cervical incompetence is a difficult one and rests essentially on a historical perspective together with investigations in between pregnancies and during the index pregnancy.
The mainstay of treatment is a cervical cerclage.

There were five babies born from this group of seven patients indicating a good prognosis for this condition. There were no complications associated with the insertion of a cerclage. The two miscarriages recorded do, however, point out that other

factors apart from simple mechanical ones play a part in the aetiology of miscarriage in cervical incompetence otherwise the treatment should have a 100% success rate. Infectious causes leading to premature rupture of the membranes and cervical dilatation is a possible cause which is reviewed later in the discussion.

## CERVICAL INCOMPETENCE

The cervix uteri is that part of the uterus which permits the growth of a pregnancy with resultant distension of the uterus until such time when it undergoes changes to deliver the pregnancy out of the uterine cavity.

When a diagnosis of cervical incompetence is made this does not follow the normal time frames and the pregnancy is delivered well before term at a time when it is often non-viable. Unfortunately, it is usually a retrospective diagnosis, as is often the case in recurrent miscarriage.

The classical description is of one or more second trimester loss which is often a rapid, pain-free delivery usually following rupture of the membranes.
An attempt at anticipating the diagnosis has been often attempted. Scores such as the cervical compliance score (197), the cervical resistance index (198) have been

described. These rely on measurement of the width of the cervical canal, hysteroscopically and by X-ray, the degree of resistance encountered to pass a Hegar 8 dilator and the degree of traction required to pass a foley catheter out of the cervix with the balloon filled with 2 ml of saline.

Embarking on these investigations will depend on a policy of routine vaginal examination during pregnancy and this has not been shown to improve outcome (199).

The use of ultrasound has, however, provided an opportunity of making a diagnosis of possible incompetence and therefore intervene before it is too late.

Use of trans-abdominal ultrasound (200) did not allow accurate measurements of the cervix as the need for a full bladder distorts the cervix.

Trans vaginal ultrasound, however, allows for accurate cervical length measurements. The ultrasound description is that funneling or beaking of the cervix, either at rest or in response to transabdominal, fundal pressure is the ultrasound diagnosis of cervical incompetence (201,202,203).

Furthermore, the shorter the cervix (normally 38-42mm) is found to be at 24 to 28 weeks gestation the greater the relative risk of a preterm delivery (204). Later studies have shown the correlation of cervical length measurements to the risks of premature delivery, especially in the presence of amniotic membranes within the cervical canal (205,206).

Most cases of cervical incompetence are congenital (207). There are, however, a number which are iatrogenic in origin. Cone biopsy of the cervix is considered an indication for cervical cerclage in a susbsequent pregnancy (208) while a study on the risks for women who have undergone LLETZ procedures was reassuring (209).

Repeated terminations of pregnancy is also considered to be a risk factor for premature delivery while a single second trimester miscarriage carries a 14% risk of a second prema-

ture birth (208).

## TREATMENT

The mainstay of treatment of cervical incompetence is surgical repair.

The 1950's saw the development of surgical techniques aimed to treat cervical incompetence. The first was the report by Lash and Lash which addressed defects in the anterior lip, presumably from previous surgical intervention (210).

The technique of cervical cerclage was described by Shirodkar first (1955) and later by Mcdonald (1957) (211,212).

Both these procedures employ a vaginal approach to repair. In Shirdodkar's technique the anterior vaginal fornix is opened and the bladder reflected upwards to exposed as much cervical length as possible and therefore place the suture as close to the isthmus as possible. This indicates that from the inception of the procedure concern that the suture be placed as high enough as possible to achieve the desired effect was present.

In McDonald's technique the procedure is a simple purse string suture using four or five bites from the cervix.

Mersilene tape is often used but may be associated with ascending infection due to a capillary effect. Nylon may have an advantage in this respect.

One further variation in technique is to place two mattress sututres at right angles to each other which may be easier to use in an emergency cerclage (The Wurm Procedure : 213)

In 1965 Benson and Durfee described the transabdominal cervical cerclage (214). This is largely reserved for failures of the vaginal approach or in the case of congenital uterine anomalies. Some cervices may also be so traumatized that vaginal repair is impossible. In this case the uterovesical space is exposed and the bladder reflected downwards, a mersilene tape is placed around the cervix below the uterine vessels away from the ureters and tied anteriorly. It is left in-situ for the woman's reproductive life. It has been advocated to perform the operation during pregnancy between 10 and 14 weeks (215). During pregnancy the space and point of insertion can be identified more readily. The suture may be removed by posterior colpotomy if it becomes necessary during pregnancy; posterior ligation of the suture is advocated for this reason (216)

The reported results indicate the validity of these techniques. In 1971 McDonald (217) reported a 94% success rate while in 1980 Cousins (218) also reported an improvement in terms of fetal survival from 21.8 to 82.5%.

There are a number of reported series of transabdominal cerclage. Two series from The Netherlands, Wallenberg and Lotgering (1987) reported 16 pregnancies in 14 patients while van Dongen and Nijhuis (1991) reported another 16 pregnancies from 14 patients. Mahran (1978) and Novy (1982) reported its use on 26 patients together from the United States while Tobiassen (1982) reported 17 pregnancies from 17 patients from Denmark. In the study by Cammarano (219) an improvement of 93% successful outcome against 18% prior to the procedure while in the fifty women reported by Gibb and Salaria (216) the improvement was of 85%. There were 9 adverse outcomes in the latter study; 4 were miscarriages before 14 weeks gestation, 3 after 14 weeks but before viability while 2 were preterm deliveries and neonatal death. Of the fifty women studied six remained childless while four cases were delivered preterm but the babies survived.

Randomized controlled trials of cervical cerclage have been attempted.

The most extensive was the MRC/RCOG trial which had two arms, cerclage and no cerclage with 647 and 645 patients repectively (220). This study has been widely criticised in that the admission criterion used was doubt on the part of the obstetrician about whether to use a suture. Inevitably this resulted in a heterogenous group of cases with a consequent weak conclusion.

The results did, however, suggest that the operation had an important beneficial effect in 1 in 25 cases in the trial (95% confidence interval). The use of cervical cerclage was associated with increased medical intervention and puerperal pyrexia. The authors concluded that cerclage should be offered to women at high risk, such as those with a history of three or more pregnancies ending before 37 weeks gestation. Dor et al (221) reported his study on twin pregnancies only while Lazar's (222) study population had a background risk of premature delivery of only 6% which makes it a very low risk group and therefore of dubious value to the whole discussion. Rush et al (223) reported on a group with a risk of 33% of premature delivery. His conclusions were that there is no beneficial effect of cerclage on length of gestation and vital outcome though these differences may be compatible with random error (224).

A more recent study is the study by Rust et al (225). Patients selected either had a risk factor for premature birth and were therefore screened by TVS or if transabdominal examination was suggestive of incompetence. A thorough examination of the length of the cervix, width of the canal and any prolapse of the memebranes was recorded. Exclusion criteria included prolapse of the membranes beyond the cervical os and or the presence of a lethal fetal or chromosomal abnormality. Furthermore an amniocentesis was performed and the fluid cultured for the presence of micro-organsims. Patients with

positive cultures were excluded from the study.
The cerclage group reported a higher incidence of chorioamnionitis (16.1%) and fewer cases of abruptio placentae (12.9%) compared to the no-cerclage group (6.6 and 16.6%). Gestational age at delivery, the incidence of preterm birth and of neonatal morbidity were found to be similar between the two groups. The survival curves constructed for the two groups were also found to be similar.

Of note is the fact that prophylactic cerclage is overused with as many as 96% being unnecessary (MRC/RCOG study 220). This reflects an inability to diagnose cervical incompetence accurately. Incompetence may be the end result of many patho-physiological processes such as infection or immunologically mediated inflammatory stimuli. Congenital weakness of the cervix has also been demonstrated using hydroxyproline (a measure of cervical collagen concentration) and extractability (a measure of collagen stability) (207).
Preliminary results from the dutch CIPRACT (cervical incompetence prevention randomised cerclage trial) have not shown any differences between the prophylactic cerclage group and the observational group in terms of preterm delivery rate <34 weeks gestation and the neonatal survival rate. In this study if patients randomised to the no cerclage group are shown to have a cervical length of <25 mm they are randomised again either to have a cerclage or conservative management. Results indicate that TVS may avoid unnecessary intervention

(226).

All these factors make cervical incompetence an enigmatic condition which will continue to be overtreated in the hope that all the pregnancies that can be saved by a cerclage will be

saved.

## CONGENITAL ABNORMALITIES OF THE UTERUS

There were ten cases of recurrent miscarraige due to congenital malformations of the uterus. Nine of these were treated with a combination of tocolytic drugs and a cervical cerclage and there were seven babies from this group, while one underwent a metroplasty after five successive miscarraiges and had a live baby at 36 weeks gestation by caesarean section.
The tocolytic drugs used were employed in sequential order, dydrogesterone, indomethacin, ritodrine hydrochloride and nifedipine retard.

Perhaps the most important name in this topic is that of Erwin O Strassmann who described his metroplasty technique and promoted it to treat recurrent miscarriage due to congenital malformations of the uterus (227).
Strassmann pointed out in 1966 that the incidence of uterine malformations reflected the physician's interest in the condition and while before that time it could only be diagnosed by bimanual examination, uterine sounds or post-partum manual exploration by that time X-ray hysterography confirmed that uterine abnormalities were comparatively frequent.
He lists seven investigators between 1937 and 1955 who reported the percentage of uterine abnormalities detected in

hysterographies; the range was 1.1 to 3.5%. In their total of 6888 hysterograms there was an incidence of 2.3%.
Strassmann states that 'in divided uterine cavities only half of the normal space is available, and many pregnancies reach a point at which the semi-uterus cannot expand further and miscarriage results. Restoring a normal uterine space by metroplasty solves the problem. I consider two consecutive miscarriages an indication for hysterography, and in a case of double uterus, sufficient indication for surgery.'

Writing in Fertility/Sterility in 1986 Alan DeCherney started his paper by stating 'an important cause of first trimester pregnancy loss is defective Mullerian fusion'. He quotes a 20% risk of reproductive problems in women with this pathology (228).
The overall incidence of uterine abnormalities has been calculated at between 0.1% (229) and 3.5% (230).

Unfortunately the situation is not so clear. Open surgical correction of congenital anomalies has been reported to be successful in terms of subsequent pregnancy outcome but may be associated with significant post-operative infertility due to the formation of post-operative adhesions (231).
The studies published on metroplasty have not been controlled and simply reported miscarriages rates before and after metroplasty. This leaves any conclusion open to criticism.

Kirk EP et al reported a series of 16 patients in 1993 (232) performed between 1972 and 1982 14 of whom achieved improved obstetric results with metroplasty. As the authors point out, would the improvement have happened with time and not required surgical intervention? In 1960 White (233) pointed out the tendency for a woman with a divided uterine cavity, history of abortion and no surgical intervention to carry longer in successive pregnancies and finally to deliver a live child. The study of Kirk et al supported this observation when the improved obstetric outcome in the surgical group after metroplasty was similar to outcomes in both the non-matched and the matched non-surgical groups, with no statistically significant differences noted.

The original classification by P Strassmann of uterine anomalies (234) identifies two main types of anomalies; the externally divided uterus with two separate uterine bodies, subclassified according to the degree of bifurcation into (1) the uterus arcuatus, (2) the uterus bicornis unicollis (bicornuate uterus), and (3) the uterus bicornis bicollis and uterus didelphys (uterus with two corpora and two cervices);and the externally unified uterus with two endometrial cavities, subclassified into (1) the uterus septus (the septum reaches the internal os), and (2) the uterus subseptus (the septum does not reach the internal os). In the asymmetric group are two main types: (1) the double uterus with one rudimentary horn , and

(2) the semiuterus with development of only one horn.

The type of uterine abnormality has been correlated to the incidence of habitual miscarriage (235) . The worst prognosis is apparently in women with a single uterine horn or a bicornuate uterus. This may reflect implantation in an area of poor vascular supply. In fact, DeCherney (236) states that only a septate uterus is associated with habitual abortion, the bicornuate uterus and uterus didelphys are often associated with a normal pregnancy outcome. Inadequate implantation and nourishment are cited as the cause of miscarriage.

The development of hysteroscopic techniques to resect uterine septa (237, 238) have the advantage of reducing hospital stay and decreasing the risk of post-operative adhesion formation with consequent infertility.

The procedure is usually performed under laparoscopic control with dextran as the distending medium in the uterine cavity. A septum of about 1cm at its broadest width can be resected in this way and this is carried down to the myometrium.

A variant of the metroplasty technique is the Thompkin's procedure.

## UTERINE FIBROIDS

Uterine leiomyomata occur in one of every four to five women in reproductive life (239, 240). They are the most common solid pelvic tumours in women.

Spontaeous abortion in women with leiomyomata may result from increased uterine irritability and contractility, either because of the tendency towards rapid growth and consequent degeneration or because of alterations in oxytocinase activity (241).

Alterations in endometrial stroma or vasculature may also be an important cause of fetal wastage associated with leiomyoma. As noted above, the endometrial venule ectasia observed by Farrer-Brown et al (242, 243) and the reduction in uterine blood flow demonstrated by Frossman (244) may in some way render the blood supply of the developing placenta and fetus inadequate, and may ultimately contribute to spontaneous abortion.

Surgical resection of fibroids in a patient with recurrent miscarriage must be attempted with due care as the same possible complication as for metroplasty i.e. post-operative infertility due to adhesions is a very likely possibility.

# CHAPTER V

# GENETIC CAUSES: CHROMOSOMAL ABERRATIONS

The analysis of aneuploidies, translocations, and other gross structural aberrations of the chromosomes has helped explain some cases of recurrent miscarriage (245, 246).
It must , however be pointed out that cytogenetic testing fails to give a complete picture of the genetic aetiologies of recurrent miscarriage.

Chromosomal aberrations are investigated by parental karyotyping from a peripheral blood sample.
This test was performed on only 32 couples in this group of patients. All had suffered three recurrent miscarriages as this was a necessary requisite to be referred for the test.
There were three positive results among these 32 patients who performed the test; in all cases this was a maternal abnormality.
The abnormalities detected were a pericentric inversion and two reciprocal translocations. This represents an incidence of 9% (for the 32 couples who undertook the test). This is certainly higher that the 3-5% incidence reported in the literature (11,59, 247).

These patients were referred for genetic counselling which was arranged by consultation with the Genetics Clinic under Professor Alfred Cuschieri.

Chromosomal **translocations** are the most common structural abnormalities associated with early recurrent pregnancy loss (248,249,250). Most carriers of a balanced translocation will be phenotypically normal and only investigation will bring it to light.

Again it may not be associated with recurrent miscarriage as Portnoi (250) showed in his study on 1142 couples where the highest incidence of chromosomal abnormalities (6.6%) was among 256 couples who had suffered one miscarriage followed by the birth of a healthy infant.

There are two types of translocations; Robertsonian and reciprocal. In the former two chromosomes adhere to each other and the total number of chromosomes is 45 though one represents two.

Studies indicate that when the Robertsonian translocation is maternal, there is a greater risk that the fetus will exhibit an unbalanced phenotype (251).

Homologous Robertsonian translocations are highly incompatible with embryonic and fetal survival, often ending in early pregnancy loss; heterozygosity may lead to partial

monosomy or trisomy and 'milder' phenotypical expression in offspring, often compatible with survival.

Another type of chromosomal aberration are **inversions;** this involves the reversal of order of genes usually as a result of two chromosomal breaks, followed by reinsertion in reverse order of the free segment. This results in either pericentric or paracentric inversions. Inversions increase the risk of fetal loss due to abnormal combination during cross-over of the meiotic loop. This results in fetal wastage, although if a balanced combination results the fetus will be normal.
Chromosomal inversions occur less frequently than translocations and a female inversion carrier is at an approximately 8% risk of producing anomalous offspring ; the risk being reduced to 4% in male carriers (252).
Regan (253) questions the role of inversions in recurrent miscarriage, especially since they are found in about 1% of the normal population.
A further aberration; ring chromosomes are a very rare situation and these are usually associated with dysmorphic individuals rather than recurrent miscarriage (254).

Peripheral blood karyotyping is an expensive test but one that is necessary in order to identify those patients who would benefit from referral for genetic counselling.
This is usually not as bleak as it may seem when one consid-

ers that there is no treatment. One third of couples with a significant abnormality will have achieved a successful pregnancy in addition to their miscarriages when diagnosed (263).

A case must be made for the karyotyping of fetal products in all cases of recurrent pregnancy loss. The information obtained will be invaluable in deciding the cause of miscarriage in all cases. Patients who have an established cause of recurrent miscarriage may still be subject to structural chromosomal anomalies due to dysjunctional events at meiosis. Identifying this will not affect judgement on a particular treatment protocol used in that case.

Contrary to what may be expected analysis of fetal material in recurrent pregnancy loss shows that these are usually chromosomally normal (255, 256).

Material from cases of spontaneous miscarriage are more likely to be abnormal, about 50-70% being abnormal (257). Warburton et al (255) have shown that there is a significantly increased risk of a chromosomally normal miscarriage after a miscarriage with abnormal karyotype.

Stern et al (256, 259) reported two studies concerning embryonic karyotypes in patients with recurrent abortions and found no difference in the frequency of abnormal karyotypes between single and recurrent aborters.

In a study on 1,309 women with recurrent miscarriage (n = 2-20) Ogasawara (260) found that the frequency of

abnormal karyotypes decreased significantly with the number of previous miscarriages while that of normal karyotypes increased significantly as the number of previous miscarriages increased. The abnormal karyotype rate did not change with the number of previous miscarriages (the mean rate was 18.3%).

This result supports the notion that a normal-karyotpye miscarriage is often associated with a subsequent normal-karyotype miscarriage; i.e in recurrent miscarriage factors other than genetic are more likely to be responsible for a repeat miscarriage.

The authors concluded that 20% of pregnancies in recurrent aborters will result in miscarriages caused by abnormal embryonic karyotypes independent of the number of previous abortions.

This means that in a case with a known cause of recurrent miscarriage, given that the prescribed treatment is **perfect,** the maximum success rate can only be 80%.

In a subsequent study Carp et al (261) investigated the incidence and type of chromosomal anomalies in the abortus after recurrent miscarriage, the prognosis for a live birth after a euploid or aneuploid abortion, the different incidence of chromosomal anomalies in primary and secondary aborters and the incidence of chromosomal anomalies correlated to maternal age.

The report was based on an attempted karyotyping on 167 abortuses which was successful on 125 of them. Of these 29% turned out to be karyotypically abnormal.
The most common anomaly was a trisomy (21 being the most common of these), followed by triploidy, monosomy X and unbalanced translocations.
This group reported a lower incidence of chromosomal anomalies than Osagawara and they explain this because their patients all had 3 miscarriages whereas the former (and also Stern's group) had only 2.
They did agree with Osagawara that patients who miscarried an abnormal fetus had a better prognosis for the future but their results did not reach significance levels. They could not comment on the type of chromosomal anomaly in recurrent abortion as the numbers who suffered this event were too small. Finally maternal age significantly changes the incidence of chromosomal anomalies, presumably due to the known association with non-dysjunction.

The finding of an aneuploid karyotype in recurrent miscarriage is reassuring while a euploid karyotype is associated with a worse prognosis.

It must be pointed out that fetal karyotyping is the only test in recurrent miscarriage that is pathognomonic; all other tests are by association alone. Also the banding technique, on

which our results are based, can only assess structural and numeric rearrangements and can fail as a result of contamination, culture failure or overgrowth of maternal cells (262).

New techniques such as comparative genomic hybridization (263) or fluorescent in-situ hybridization (264) make possible the analysis of tissue that cannot be cultured.

The role of single-gene mutations, X inactivation and imprinting effects (differential expression of genetic material) will hopefully become clearer as new techniques based on molecular analysis become available. Our knowledge on the genetic contribution to recurrent miscarriage will then become much more complete and possibly a more important role will be assigned to this cause of recurrent miscarriage.

# CHAPTER VI

# INFECTION

One of the classical tests in the workup of a patient suffering from recurrent miscarriage is the TORCH screen. This refers to antibody testing for the presence of toxoplasma, rubella, cytomegalovirus and herpes.

All these organisms may cause miscarriage in a mother with a primary infection. However, any immunocompetent patient will produce antibodies and protect her from any adverse effects in a future pregnancy.
The general view is that most couples do not benefit from an extensive infectious work-up and the routine use of a TORCH screen is no longer recommended (265 and RCOG Greentop Guidelines 2000).

Bacterial vaginosis has been associated with preterm labour and second trimester miscarriage (266).
In this study by Hay where this association was described it was noted that those women in whom bacterial vaginosis was detected at sixteen weeks gestation there was a five-fold increase in the risk of mid-trimester miscarriage and a three-fold increase in the risk of preterm birth.
A more recent study found a strong association between the

presence of bacterial vaginosis diagnosed at less than fourteen weeks gestation and subsequent early pregnancy loss (267). In this study it was noted that women who suffered a miscarriage, a higher number had a history of previous early pregnancy loss compared to those with a normal pregnancy outcome raising the possibility of an association between recurrent bacterial vaginosis and recurrent spontaneous miscarriages. In another study Ralph et al found that the relative risk of a first trimester miscarriage in the presence of bacterial vaginosis was 2.03 (268).

There is now sufficient evidence to implicate bacterial vaginosis in spontaneous premature rupture of the membranes and preterm labour and its treatment has been investigated in a number of trials.

Previously bacterial vaginosis was not shown to be associated with recurrent first trimester miscarriage but the incidence of bacterial vaginosis was higher in patients who had suffered one second-trimester loss (269).

The role of bacterial vaginosis in recurrent miscarriage is still a matter of research, but in view of the results reported by Ralph et al and the other evidence it would seem opportune to start screening women attending our miscarriage clinic for bacterial vaginosis.

# CHAPTER VII

# CONGENITAL THROMBOPHILIAS

Since the Miscarriage Clinic started operating an exciting new field of research has been discovered in the field of Congenital Thrombophilias.

The term thrombophilia refers to those disorders in which there is a predispostion to thrombosis due to abnormally enhanced coagulation (270).

The role of coagulopathy in the pathogenesis of recurrent miscarriage first came to the fore with the description of the Antiphospholipid Antibody Syndrome (Hughes' Syndrome). This is due to an acquired thrombophilia.

It was only a matter of time before other hypercoaguable states became implicated in recurrent miscarriage (271) and today a proper work-up for recurrent miscarriage must include a full thrombophilia screen; i.e. the acquired anticardiolipin antibodies and lupus anticoagulant and the congenital, protein S and C deficiency, antithrombin III deficiency, Factor V (Leiden) and II mutations and hyperhomocysteinaemia.

At least five studies have implicated activated protein C

resistance in the aetiology of fetal loss (19, 272, 273, 274, 275) and this confirms the importance of testing for this condition in recurrent fetal loss and also adverse pregnancy outcome.

However, further trials (276, 277, 278) did not report significant differences in the incidence of miscarriage in patients with congenital thrombophilia and controls.

Since thrombophilia seems to be more strongly associated with mid-trimester miscarriage, it has been suggested that first trimester miscarriage is likely to be an implantation problem while mid-trimester miscarriage is due to a thrombotic event affecting the feto-placental circulation (287).

It is however, premature to put congenital thrombophilias on a par as the Antiphospholipid Anbtibody Syndrome as the incidence of these thrombophilias in women having normal pregnancies is at present unknown.
The presence of a past thrombotic event will definitely make thromboprophylaxis in a subsequent pregnancy essential.
There have been no controlled trials of the treatment of congenital thrombophilias in recurrent pregnancy loss and the practice of applying the same protocol as the APS syndrome may not stand the test of a rigorous prospective, double-blind randomised trial.

However, as everyone in this field is well aware this type of study in recurrent miscarriage is very difficult to conduct.

# CONCLUSIONS

# CONCLUSIONS

This is the first descriptive study of the problem of recurrent miscarriage in Malta.

The study group of 135 patients who were enrolled for this thesis is a representative group of the maltese population as can be judged from their ages, medical histories and diverse occupations.
This group of patients provided the full diversity of causes of recurrent miscarriage as described in the literature at this time.

There were no particular differences in the incidence of the individual causes of recurrent miscarriage and the pattern of the condition is similar to that reported in the literature.

This confirms the need for physicians who are caring for these patients to be fully aware of all the possible causes and also the treatment options that are available.
It is my belief that the 81 babies who were born to the study group were the result of the intensive and detailed care these patients had access to, through the miscarriage clinic.
This excellent result justifies the concept of a specialized miscarriage clinic.

The analysis of this study group confirmed the importance of maternal age as a risk predictor of pregnancy outcome. Advanced maternal age was statistically significant as a risk factor for miscarriage.
This must always be emphasised when counselling women with regard to planning a family.

Other factors which resulted in a significant odds ratio were an elevated follicle stimulating hormone level and a short menstrual cycle. These are probably due to advanced maternal age and are therefore an expression of the same problem. It has also been postulated that a raised FSH level may reflect poor quality oocytes with inherent chromosomal compromise (279). Again, raised LH levels may cause an erratic follicular development with a consequent effect on the resumption of meiosis leading to an increased rate of non-dysjunction, much the same way as was postulated for PCOS (54).

Further factors were the use of clomiphene citrate and an ultrasound report of a congenital uterine anomaly.
That the use of clomiphene citrate should achieve a significant odds ratio is interesting in that it probably relates to that group of patients who have a combined sub-fertility and miscarriage problem. Typically this is best exemplified by the PCOS group. However, other anovulatory cases manifested by luteal phase defects also may present with this clinical his-

tory. There were three cases where a history of recurrent miscarriage who subsequently evolved into infertility which was resistant to treatment with ovulation induction. These patients were strongly androgenic and clomiphene did not achieve the necessary stimulation.

However, in cases of luteal phase defect, clomiphene had an excellent record and its use is to be strongly recommended in these cases.

In PCOS, the severity of the endocrinological manifestation seems to be the determining factor whether clomiphene is beneficial or not. Certainly, in this group the use of clomiphene was associated with a poor result although, of course, the numbers were small (5 patients).

These endocrine factors require further evaluation in order that their exact significance can be determined with certainty and provide ideas for further research into the subject.

The finding of a congenital uterine anomaly on ultrasound examination indicates a strong association with recurrent miscarriage. This refers to a severe anomaly i.e. at least a bicornuate uterus. The application of a protocol of tocolytics though pregnancy changing from trimester to trimester the more appropriate drug, proved highly successful and its use is to be recommended.

It is to be noted that this protocol was originally designed for

patients with high-order multiple pregnancy in order to prevent preterm delivery.

The introduction of screening for bacterial vaginosis is to be recommended as knowledge on the prevalence of this condition in Malta is lacking and its possible role in recurrent miscarriage yet to be confirmed.

A thorough knowledge of the up-to-date recommended treatment protocols is essential in these difficult cases.
This is especially so as in many cases present treatment options are not very effective and new approaches are being attempted.
Furthermore there are exciting advances in what has been termed as 'reproductive immunology' which are linking the role of hormones (especially progesterone) directly to immunological processes which determine the outcome of a pregnancy.
The ever expanding field of congenital thrombophilias and their relationship to recurrent miscarriage is also a matter of debate and one that has to be followed closely.

In conclusion a recommendation for the future is the setting up of an 'early pregnancy assessment unit'. This concept implies the presence of a unit, possibly an overlap with the miscarriage clinic whereby patients with a past history of mis-

carriage are offered the possibility of a rapid evaluation by specialist care in cases of bleeding in a subsequent pregnancy (280).

The availability of this service together with the support of a trained miscarriage nurse who can take over some of the counselling and support so important in this condition, will bring the miscarriage clinic in line with the best that are available.

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