

**EPIDEMIOLOGY AND MECHANISMS OF SPORADIC AND
RECURRENT SPONTANEOUS ABORTION**

by

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ABSTRACT

There is no prospective data available on the incidence of sporadic and recurrent spontaneous abortion which the clinician can use to assess a woman's risk of recurrence, the factors predisposing to abortion and the benefits of treatment.

The incidence of spontaneous abortion in 412 women, recruited prospectively from the general population, was 12%. The risk of abortion was influenced by the previous reproductive history, the most important predictive factor being a previous history of abortion.

The possibility that an immunological cause might account for these findings was investigated by determining the incidence and natural history of anti-paternal cytotoxic antibody (APCA) in 306 of these pregnancies. The presence of APCA in the serum prior to pregnancy did not confer protection from spontaneous abortion. The development of a positive APCA test during pregnancy occurred in a minority of the patients, being rarely demonstrable before 28 weeks gestation and usually disappearing between pregnancies. These findings suggest that the absence of serum APCA are unlikely to be causally related to recurrent spontaneous abortion.

The incidence of abortion among 200 couples with a history of recurrent abortion was 34%. In this population, immunisation treatment with paternal white cells did not improve pregnancy outcome. However, these patients demonstrated several characteristics which distinguished them from patients with sporadic abortion. A history of relative infertility and a delay in conception prior to the studied pregnancy were associated with a particularly poor pregnancy outcome.

This association was further investigated by measuring luteinising hormone (LH) concentrations in 193 women before pregnancy. High follicular phase LH levels ($>10\text{iu/l}$) were a significant risk factor for spontaneous abortion. These data indicate that follicular phase LH estimations might be a useful test to assess the prognosis for a pregnancy.

The results presented in this thesis demonstrate that pregnancy outcome can be predicted by a woman's reproductive history and her pre-pregnancy LH concentration. It is possible that these two factors are causally related and that many cases of spontaneous abortion are mediated by an endocrine abnormality of the ovary which is potentially remediable.

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CHAPTER 1.

INTRODUCTION

Introduction.

1.1. Definitions and terminology.

The term "abortion" is usually used to describe the termination of a pregnancy before the fetus has reached a gestational age compatible with extra-uterine viability. Viability may be defined in terms of gestational age (duration of pregnancy from the time of the last menstrual period LMP) or the weight of the fetus or the length of the fetus. Until recently, there has been no consensus of opinion on the criteria used to assess whether or not a fetus is viable. The World Health Organisation's survey (1970) of the official definitions of abortion in 86 countries found that 38 countries had no definition and the remainder used a variety of criteria either singly or combined. Some considered abortion to signify a pregnancy terminating before 16 weeks gestation, others before 28 weeks. One country used the term 'abortion' to denote certain types of term delivery, whilst several countries employed physiological criteria, three used fetal length and one used fetal weight.

In Great Britain the legal definition of fetal viability is currently set at 28 weeks gestation, corresponding to a fetal weight of approximately 1,000 grams (The Infant Life (Preservation) Act 1929). The Royal College of Obstetricians and Gynaecologists' report on Fetal Viability and Clinical Practice (1985) recommended that the gestational age of fetal viability should be reduced to 24 weeks, but at the present time legislation has not been enforced. Subsequent to 28 weeks gestation a dead fetus is classified as a stillbirth. A fetus of any gestational age or birth weight demonstrating signs of life (respiratory movement, heart or umbilical cord pulsation, voluntary muscle movement) is defined as a liveborn infant. Death of a liveborn infant during the first seven completed days (168 hours) of life is termed an early neonatal death.

These definitions are based on the dated observations that an infant with a gestational age of 28 weeks or less and a birth weight of less than 1,000 grams has little chance of survival. Advances in neonatal care and expertise have reduced significantly (and will continue to reduce) the lower limit of fetal viability. Recognition of this fact prompted reappraisal of the definition of abortion by the WHO in 1977 whose report concluded that an abortion is:- "the expulsion or extraction from it's mother of a fetus or an embryo weighing 500g or less (approximately equal to 20 to 22 completed weeks of gestation) or an otherwise product of gestation of any weight and specifically designated (e.g.Hydatidiform mole) irrespective of gestational age and

whether or not there is evidence of life, and whether or not the abortion was spontaneous or induced."

The WHO further recommended that all fetuses and infants delivered weighing 500g or more be reported in the country's statistics. They defined stillbirth as:- "the process of birth of a fetus weighing more than 500g when there is no evidence of life after birth". Recognising that legal requirements in many countries may set different criteria for registration purposes, they advised that mortality statistics reported for purposes of international comparison should include only those born weighing 1,000g or more.

These definitions assume that infant viability can be determined by birthweight and that no infant weighing 500g or less can survive. Although the literature contains anecdotal exceptions to this assumption, at the present time, the WHO definitions of 1977 remain valid for the vast majority of immature infants with a birthweight appropriate for their gestational age. As the incidence of abortion reaches its lowest level between 20-27 weeks (Shapiro & Bross 1980, Huisjes 1984), most epidemiological data will correspond reasonably well to the WHO definition.

Hence, in this thesis, spontaneous abortion will be defined as the spontaneous loss of a pregnancy before 20 weeks gestation and does not include ectopic gestations. The term "miscarriage" is used synonymously with spontaneous abortion (Beard et al 1985). Induced abortion is defined as an abortion initiated voluntarily with the intention to terminate a pregnancy and includes terminations for fetal abnormality. Pregnancy losses occurring between 20 - 27 completed weeks, will be considered separately as late perinatally related abortions (LPRA) (Whitfield 1986) and will include abortions induced during this same gestational period because of intrauterine death or maternal medical disorders.

1.2. Frequency of abortion.

1.2.a. Magnitude of the problem.

The true frequency of early pregnancy loss is uncertain. Although it is generally accepted that 15% of clinically recognised pregnancies end in spontaneous abortion (Warburton & Fraser 1964), unless assays of human chorionic gonadotrophin (hCG) are used, pregnancy is not routinely recognised until 5-6 weeks after the last menstrual period. Until recently, standard pregnancy tests did not permit accurate diagnosis before 7-8 weeks

gestation, and obstetricians would most frequently make the diagnosis of pregnancy after the second missed menstrual period (at 8 weeks gestation and 6 weeks embryonic development). Hence, the number of studies taking into account "subclinical" pregnancy losses before 6-8 weeks gestation are limited.

Fecundability, the probability of successful pregnancy per menstrual cycle, has been reported to be approximately 25% in several epidemiological surveys conducted within communities where no contraception is practised (Short 1976, Vessey et al 1976). A major factor contributing to fecundability is the loss of embryos after fertilisation has occurred, not all of which may be recognised. Roberts and Lowe (1975) used a mathematical model to suggest that 78% of fertilisations fail to result in a live birth. Of this total predicted pregnancy loss of 78%, 2% were perinatal and 10-25% were recognised abortions, leaving a 50% loss that occurred before the clinical diagnosis of pregnancy.

The development of highly sensitive assays of β -HCG, to detect the presence of an embryo, and their application firstly as a research tool and more recently as "over-the-counter" pregnancy testing, has confirmed that the incidence of pregnancy loss between implantation and the clinical identification of pregnancy is indeed considerable, probably in the region of 60%. The incidence of clinical spontaneous abortion would seem to represent just the tip of the iceberg of total reproductive loss. Hence, a study of the frequency of spontaneous abortion must distinguish clearly between the separate contributions made by both subclinical and clinical pregnancy losses.

1.2.a.i. subclinical abortion.

Pregnancy loss occurring during the period between fertilisation, implantation and the clinical identification of pregnancy is currently defined as "preclinical, subclinical, occult or biochemical" abortion. Clinically, menstruation may be delayed for a few days, the woman may notice a slightly increased blood loss, or the menses may be perceived as normal.

Calculations show that about 15% of the eggs of healthy fertile women are lost before fertilisation. Hertig et al (1959) in their analysis of 34 fertilised ova recovered from 211 hysterectomy specimens, estimated the probability of conception to be 85%. Of these fertilised ova 15% would degenerate before implantation and a further 30% after implantation. Based on Hertig's morphological data and the clinical data of French and Bierman (1962), Leridon (1977) predicted a 69% pregnancy loss rate, with 40% occurring

between implantation and the clinical diagnosis of pregnancy. These incidences are substantially higher than any demographic estimates of the overall incidence of abortion and provide the foundation for the concept of subclinical abortion. Extrapolation of these results to other populations has led to widespread acceptance that many pregnancies are lost before becoming subjectively or objectively apparent.

Several prospective studies have estimated the incidence of early pregnancy loss on the basis of assays for hCG with variable results. The chorion begins to secrete hCG from day 6 of embryogenesis (Simpson 1981), has been detected in vitro by day 7 or 8 (Fishel et al 1984) and may be found in maternal serum or urine after that time. Miller et al (1980) measured urinary β -hCG on alternate days of the cycle on or after day 21. The criteria used for a diagnosis of pregnancy were chosen empirically, either a single value of 5ng/ml (50m-i.u./ml) or as 2 or more values above 2ng/ml (20m-i.u./ml). In 152 cycles, β -hCG was elevated sufficiently to justify the diagnosis of pregnancy. In only 102 (67%) was pregnancy appreciated clinically. The overall incidence of pregnancy loss was 42% (64/152). The pre-clinical loss rate was 33% (50/152) and the clinical loss rate 14% (14/102). Using a radioimmunoassay, Edmonds et al (1982) defined any urinary hCG value above 56 m-i.u./ml after day 21 of the cycle as indicative of pregnancy, on the grounds that this was the upper limit of the range observed in a group of sterilised women. The overall pregnancy loss rate was 62% (73/118). Only 51(43%) of the 118 positive β -hCG cycles showed clinical evidence of pregnancy. Of these 51, 6 (12%) experienced clinically recognised abortions, whereas the pre-clinical loss rate was 58% (67/118). This report highlighted the finding that 92% of all the pregnancy losses occurred without the knowledge of the mother. In contrast, Whittaker et al (1983) recorded an overall pregnancy loss rate of 20% (18/92) of which only 39% of the total number of conceptions were subclinical. Using a β -hCG serum radioimmunoassay they measured single samples obtained "about day 25" in the luteal phase, and diagnosed pregnancy if the β -hCG was 16mU/ml or greater. In this study, the clinically recognised loss rate was 13%, very similar to that found by both Miller and Edmonds, but the pre-clinical loss rate was considerably lower (8%).

The variable results from these 3 studies emphasise the difficulties in β -hCG interpretation at low concentrations. The hormonal specificity of glycoprotein hormones is determined by their β -chain. The β -chains of Luteinising Hormone and hCG share 89 of their first 115 amino acids (Tyrey &

Hammond 1976). Cross reactivity between LH and β -hCG can result in erroneous measurements of LH, particularly important when the assay attempts to measure very small amounts of β -hCG. Designating a very low level of HCG as evidence of pregnancy will almost inevitably result in a spuriously high loss rate, some women with a normal menstrual period being falsely diagnosed as having had an early fetal loss. Conversely, requiring only a high β -hCG level as diagnostic of pregnancy will result in a low loss rate. True pregnancies ending in early abortion might never generate sufficient β -hCG for identification, especially if abnormal (Lenton et al 1982).

Recognising that the discrepancies in previous estimates of the incidence of early pregnancy loss may be due to differences in the specificity and sensitivity of the methods used to diagnose a pregnancy, Wilcox et al (1988) measured urinary hCG using an immunoradiometric assay capable of detecting hCG levels as low as 0.01ng/ml, with virtually 100% specificity for hCG in the presence of LH. Their criterion for early pregnancy (an hCG level above 0.025ng/ml on three consecutive days) was determined by comparing hCG levels in the 221 women in the study group with 28 women who had undergone sterilisation by tubal ligation. Increases in hCG levels near the expected time of implantation identified 198 pregnancies and the incidence of clinically unrecognised early pregnancy loss was 22% (43/198) and the total rate of pregnancy loss after implantation, including clinically recognised spontaneous abortions was 31%. The assay used in this study is able to detect only those pregnancies in which intact hCG was produced and reached the urine. Nonetheless, most of these early pregnancy losses would not have been detectable by the less sensitive assays for hCG used in the previously cited studies.

These studies of the frequency of biochemical pregnancy cited earlier used menstrual history to define the date of ovulation and interpreted elevated hCG values on or after day 21 of the cycle as evidence of pregnancy. The recent study of Walker et al (1988) concluded that without accurate knowledge of the timing of ovulation it is not possible to exclude the possibility that transient rises in hCG after day 20 of the menstrual cycle were due to cross-reaction with late ovulatory surges of LH. Using antisera of both low and high specificity for the estimation of hCG secretion, together with an LH assay, these workers collected daily urine samples from day 5 of the cycle until menses, in normal fertile women. They noted that the mid-cycle elevation of LH is more prolonged in urine than in serum, ranging from 2 to

7 days, and that ovulation occurred after day 18 in 21 of the 75 cycles studied (28%). Late ovulation would have been predicted on the basis of menstrual history alone in only 11 of these 21 cycles. If the date of ovulation had been predicted by menstrual cycle length and only the low specificity antiserum used in the assays, biochemical or occult pregnancies would have been diagnosed in 14% of the cycles. These hCG rises were not confirmed when the urine samples were retested with the higher specificity antiserum, leading these authors to conclude that previous studies have substantially overestimated the frequency of clinically unsuspected biochemical pregnancy.

However, any estimate of early embryo loss derived from hCG or β -hCG assays cannot include those embryos who fail to reach the stage of hCG secretion. Surveillance of embryo loss during the period between conception and blastocyst formation requires some other means of identification. Measurements of early pregnancy factor (EPF), which appears in maternal serum within 24 hours of conception and persists for as long as the conceptus is viable, demonstrate that 75% of conceptions are lost in the first 14 days (Chen et al 1985, Rolfe 1982), thereby confirming the theoretical suggestions of Roberts and Lowe (1975).

Early pregnancy associated thrombocytopenia has now been observed in women within 24 hours of embryo transfer after InVitro fertilisation (O'Neill et al 1985). The conceptual product responsible has been termed embryo-derived platelet activating factor (EDPAF). Assays of EDPAF in the immediate post-ovulatory phase of the menstrual cycle may provide a means of assessing very early embryo loss in a normal population (Spinks 1987).

In summary, subclinical pregnancy losses clearly occur but their frequency remains uncertain. Based on the combined use of EPF and β -hCG (which becomes positive after implantation) it can be estimated that of 100 fertilised embryos, 20-30 will not implant, 20 will attempt to implant (single low level spike of β -hCG like material) and 5-10 will abort after the clinical diagnosis of pregnancy is made (Clark 1988). The proportion of early embryos lost may vary with the population studied. In older patients and in infertile couples the occult abortion rate may be higher (Sharp et al 1983, Romeu et al 1987), whereas in other groups the total early loss may be as low as 25% (Fleming et al 1984). The increasing demand for assisted fertility treatments, the success of which are judged by the number of successful implantations achieved, will undoubtedly mean that the incidence of occult pregnancy

amongst women of normal and subnormal fertility will continue to attract considerable attention.

1.2.a.ii. clinical abortion.

The incidence of clinically recognised spontaneous abortion is generally quoted as 15% (Warburton & Fraser 1964). This figure is surprisingly consistent, considering the wide variations in the populations available for study, methods of recruitment and the type of data analysis employed. Indeed, the factors and variables that may affect the empirically derived incidence of spontaneous abortion are so numerous and diverse, that it has been suggested that there is no normal incidence of spontaneous abortion that can be applied to all women (Jansen 1982a). They can be usefully divided into three main categories:-

- (1) Variables of established importance - such as maternal age, gravidity and a previous history of abortion. The effects of these important variables are considered more fully below.
- (2) Observational errors - relating to the precise diagnosis of spontaneous abortion either clinically or historically. In this category, the under-reporting of subclinical abortion is of particular importance and has already been considered in detail. Interestingly, the converse situation of sporadic delayed menses (isolated anovulatory cycles or ovulatory cycles with a prolonged follicular phase) has received little attention in epidemiological studies. The contribution of induced abortions mislabelled spontaneous is difficult to assess, but must be considered as a further source of inaccuracy, particularly in studies performed before the introduction of legislation for therapeutic abortion.

In contrast to these established variables and problems of observation, which are applicable to all studies, there are in addition:-

- (3) Artifactual variables - reflecting the bias introduced by the design of the individual study. For example, "memory bias" may be significant in retrospective studies dependant on patient recall of past events (Yerushalmy et al 1956, Axelsson & Rylander 1982). "Reproductive compensation bias" may affect the frequency of abortion, depending on whether the outcome of a previous pregnancy motivates a woman to persist (James 1963, Naylor 1974) or to desist from attempting further pregnancies (Leridon 1976). However the most important bias is introduced by "patient selection" and the sampling methods employed for the study population. The incidence of early

spontaneous abortion is likely to be underestimated in reports based on hospital populations since symptoms are lighter and the patient less likely to seek specialist help.

1.2.a.iii. Variations in the frequency of abortion in previous studies.

Retrospective studies of pregnant women are plentiful, since this type of study design (using a pregnancy to ascertain previous pregnancy outcomes) is an efficient and quick way of reaching a large proportion of the potential sources of data. An overall abortion incidence of 14.7% was noted in the study by Warburton and Fraser (1964), forming the basis for the widely held belief that 15% is the normal incidence of spontaneous abortion, although the incidence in the larger study by Naylor and Warburton (1979) was 12.6%. However, these studies will included pregnancies that had reached the gestational age required for entry. This bias can be minimised by considering for analysis only previous pregnancies and excluding the current "recruitment" pregnancy (Naylor 1974).

Retrospective studies of non-pregnant women avoid selection bias, but have a much lower yield of data. In the few available studies of this type, misrepresentation of induced abortions and patient memory recall are conspicuous problems (Tietze & Martin 1957, Leridon 1976).

Prospective studies that recruit patients who are already pregnant are strongly influenced by the special interests of the clinician to whom the patients are referred. They are further biased by the exclusion of early abortions that did not require any medical intervention. Consequently estimates of the incidence of abortion from these studies are low. For example, the Norwegian study carried out by Petersson (1968) documented an incidence of 9.8%. Harlap's study (1980) recruited 32,000 women during the first two trimesters of their pregnancy and recorded an overall abortion rate of only 4.7%. Using life table analysis however, the estimate was that 14.4% of the women observed from 5 weeks onwards would abort before week 28 of pregnancy. The application of life table methods to prospective data (which gives rates per pregnancy period and corrects for the loss of women to follow up who abort at an earlier stage in gestation) produces another bias. Symptoms of possible loss make early presentation of a pregnancy more likely, and early embryonic or fetal loss is exaggerated unless women who present with symptoms of threatened abortion or who abort within a short interval after entry to the study are excluded (Shapiro et al 1971)

The ideal study recruits non-pregnant women of reproductive age and observes their subsequent pregnancies prospectively. The study of Roth (1963) has the added advantage that it was based on 3,500 women personally treated by the author between 1945 and 1962; he found an overall incidence of 15.6%. French and Bierman (1962) surveyed the entire island of Kauai, and then attempted to document every subsequent pregnancy. Only 19% of the 3000 pregnancies were recruited before 8 weeks gestation and the incidence of spontaneous abortion was 9.1% before life-table manipulation of the data, when the figure rose to 23.6%. An overall figure of 17% was noted by Reed and Kelly (1958) who interviewed 150 couples engaged to be married and documented their incidence of spontaneous abortion by conducting a second interview 20 years later. More recently, the Royal College of General Practitioners (1976) reported a study on pregnancy outcome after oral contraceptive usage, noting the incidence of spontaneous abortion in their control group to be 13.4%.

In summary, there are very few studies available documenting the incidence of spontaneous abortion in a normal, representative population of pregnant women. In order to avoid the most important types of selection bias the study design needs to be prospective, recruiting subjects from the community prior to pregnancy. At the present time, the majority of data on the incidence of spontaneous abortion are from patients undergoing treatment for infertility, since infertile couples come under close periconceptual observation and their resultant pregnancies are invariably well documented.

1.2.b. Gestational age distribution of spontaneous abortion.

The risk of spontaneous abortion varies with the gestational age of the fetus. A peak in the distribution of clinical spontaneous abortions occurring around 8-12 weeks was clearly demonstrated by Stevenson et al (1959), who observed that 45% of losses occurred between gestational weeks 7 to 11, and 30% between weeks 12 to 15. Very few losses were recognised before 7 weeks. These findings were confirmed by several other studies (French & Bierman 1962, Erhardt 1963, Petersson 1968, Shapiro et al 1971).

The realisation that the frequency of fetal losses are related to gestational age has led to the widespread application of life-table analyses to calculate the probability that an abortion will occur during successive weeks or months of pregnancy. Two of the most frequently quoted studies estimated

the overall loss rate at 20 weeks gestation to be 23.6% (French & Bierman 1962) and 21.6% (Shapiro et al 1971). The estimated loss rates in the 8-11 week interval were 7% and 8.1% respectively.

However all these studies were based on clinically recognisable fetal losses usually after 8 weeks gestation. More recently, the use of ultrasound has rendered gestational interval studies both invalid and obsolete. Early pregnancy failure can now be diagnosed in the absence of any overt clinical signs on the basis of ultrasound criteria alone, namely, absence of fetal heart activity and crown-rump length appropriate for gestational age. Several studies now reveal that a viable 8 week old fetus has a 96-98% likelihood of survival (Simpson 1984, Christianens & Stoutenbeek 1984, Wilson et al 1984). Spontaneous abortions recognised clinically during the 8-12 week interval are more frequently pregnancies in which fetal death occurred much earlier (missed abortion).

That the vast majority of pregnancies ending in clinical spontaneous abortions have already failed by the 8th gestational week of pregnancy suggests that other variables thought to affect the incidence of spontaneous abortion by gestational interval need to be reassessed. For example, any attempt to correlate specific chromosomal abnormalities with gestational interval is inadequate without using ultrasound criteria for the diagnosis of fetal death. Whether chromosomally normal fetuses are retained in utero for longer than abnormal chromosome complements, or alternatively whether the interval of missed abortion is comparable in both groups and for different types of abnormality, is not understood.

1.2.c. Maternal age.

The association between rising maternal age and the incidence of spontaneous abortion has been well documented in cross-sectional studies (Shapiro et al 1971, Stevenson et al 1959, Stein et al 1980, Shapiro & Bross 1980). This finding has long been accepted as a reflection of the increased risk of some fetal chromosomal abnormalities (trisomies) and neural tube defects with rising maternal age. As with any study of early fetal loss, the data only relates to those abortions which are both recognised and reported. All the caveats concerning study design and bias discussed earlier (1.2.a.ii) apply to this important variable, particularly at the earliest gestational ages, where the available data are known to be incomplete.

The morphological study reported by Fantel et al (1980) noted a deficit of abortions in young mothers and an excess in mothers aged 30 years or more, but probably represented less than a third of all fetal losses in the population studied. Harlap et al (1980) found a J-shaped pattern of risk, an initial rise before the age of 18, little change until the age of 35 years, following which, a sharp rise in the incidence of spontaneous abortion was evident. Several other studies have reported similar results (Stein et al 1980, Shapiro & Bross 1980, Stevenson & Warnock 1959). The increased incidence of fetal loss within younger age groups may in part reflect the social problems often experienced by young mothers, in addition to a biological age effect. In recent years, the increasing numbers of patients undergoing assisted fertility treatment and pre-natal diagnosis, in which evaluation and counselling of pregnancy loss are crucial, has further helped to demonstrate a clear age-related pattern of abortion. Gilmore and McNay (1985) evaluated the results of ultrasound scans performed on 2139 patients who booked for antenatal care before 11 weeks gestation. They demonstrated that the percentage of non-viable pregnancies (anembryonic gestation sacs and missed abortions) was higher over the age of 39 years (15.4%) compared to an average for all age-groups of 8.4%. The comparison was even more striking after fetal viability had been confirmed by ultrasound, when the fetal loss rate was 13.6% over the age of 39, compared to an average loss of 1.9%.

The factors which influence maternal age at reproduction are complex and include age at menarche and menopause, desired family size and pregnancy spacing, all of which may be affected by cultural and socio-economic circumstances. What is not clearly understood is whether it is these factors, rather than maternal age alone which determine the risk of fetal loss. Those most likely to be closely associated are pregnancy order (a variable inextricably linked to maternal age) and pregnancy spacing (both very close spacing and involuntary infertility).

1.2.d. Gravidity and pregnancy order.

In order to separate out the independent effects of age, gravidity and pregnancy spacing, several studies have looked at the risk of early fetal loss in pregnancy sequences within the same women (Billewicz 1973, Bakketeig & Hoffman 1979, Pharoah et al 1977, Roman et al 1978). Because of the methods of data collection used for these sequential pregnancy studies, the analyses have to be within gravidity or birth order groups, with age as a subsidiary

variable. Perhaps the most useful is the data obtained from a group of women doctors, selected because they were on the medical register in 1975, and who were first registered in 1950 or later (Roman & Alberman 1980). Although these women may be atypical in their reproductive behaviour because of the demands of their careers, they are more likely to remember details of past pregnancies and to recognise early pregnancy losses.

Their curve of fetal loss rose after the second pregnancy with increasing pregnancy order, and therefore by implication with rising maternal age. However, when the women were ranked by gravidity as well as pregnancy order the fetal loss rates appeared to fall and not rise with increasing pregnancy order. Similar results were reported by Billewicz (1973) and Bakketeig & Hoffman (1979). The interpretation of these results has led to considerable debate (Golding et al 1983, Roman 1984). The chances of a woman being in a specific gravidity or pregnancy order subgroup depends on many factors, in particular her desired family size and previous reproductive outcome. The wish to compensate for a previous pregnancy loss may motivate a woman to continue her attempts to produce a healthy child until she achieves her aim (James 1974). In our Western society, where the average family size is two children, the high pregnancy order subgroups are likely to include women who have had previous pregnancy failures. This factor probably accounts for much of the apparent rise in fetal loss seen with increasing gravidity, if pregnancy order is held constant. The last pregnancy embarked upon is the least likely to be a fetal loss, since a woman is more likely to stop with a success than a failure. Hence a large fall in the abortion rate within gravidity groups is observed between the penultimate and the last pregnancy. The tendency to abandon further attempts at pregnancy after a successful pregnancy may produce another artefact, giving the false impression that the rate of spontaneous abortion is falling with increasing birth order, and by implication, with maternal age.

1.2.e. Primigravidae.

Recognising that gravidity is a complex variable and fails to distinguish between women with widely differing obstetric histories, it has been suggested that the independent effect of maternal age on fetal loss rates would be best studied by examining first pregnancies, in which the picture is not confused by previous pregnancy outcomes. The literature lacks prospective data for the incidence of spontaneous abortion in normal primigravidae, but retrospective

studies have estimated figures of 8-11% in women under the age of 30 years, increasing to 15-23% after the age of 35 years (Naylor & Warburton 1979, Shapiro et al 1971). Unfortunately, more recent attempts to establish the incidence of spontaneous abortion in primigravidae have centred mainly around infertility patients, whose mean age is not representative of the normal population.

Using the retrospective data set of first pregnancies from the women doctors study, Roman (1982) confirmed the J-shaped curve of spontaneous abortion with increasing maternal age. The mean age of women whose first pregnancy ended in an abortion was higher than primigravidae who had live births. The increased risk of abortion at low maternal ages persisted until the mid-twenties and the rise with older age groups began in the mothers having their first pregnancy at the age of 38 years, following which a progressive increase in risk was recorded, although the numbers involved were small. Since this study group was composed of professional women, who had tended to postpone their first pregnancy (modal age 27-28 years), it is possible that those who embarked upon a pregnancy in their early twenties were different from the rest of their peers.

After the first pregnancy, the effect of maternal age on the incidence of abortion becomes further complicated by the outcome of the first pregnancy, the age at that pregnancy and the interval before the subsequent pregnancy. As gravidity increases, the mean maternal age at first pregnancy falls. Women who have large numbers of pregnancies are younger at the time of conceiving their first pregnancy than women who subsequently have fewer pregnancies (Roman 1982). Furthermore, the time interval before an abortion tends to be longer than the interval before a livebirth in women of the same pregnancy order and gravidity group, suggesting that the association of increased maternal age with fetal loss may be secondary to problems of sub-fertility. Nonetheless, the tendency for maternal age to be higher at the time of a miscarriage remains, since women whose first pregnancy ends in a livebirth are younger than women whose first pregnancy miscarries but when this livebirth is followed by one or more abortions, such women are older at the time of conceiving their first child when compared to women who experience no miscarriages (Alberman 1987).

In summary, the relationship between early fetal loss and maternal age is complicated by variables such as gravidity and previous pregnancy outcome. The risk of spontaneous abortion increases with maternal age after the mid-

thirties and has been most convincingly demonstrated in first pregnancies, before the picture has been confused by attempts at reproductive compensation. Very low maternal age may also be a risk factor, but this possibly reflects the social characteristics of this group in addition to a biological age-effect. It is not possible to conclude from the data whether the adverse association of age on the incidence of abortion is causal. It may reflect problems of fertility, resulting in longer interpregnancy intervals, and vary according to the karyotype of the abortion as will be discussed in the next section.

1.3. Investigation and treatment of established causes of spontaneous abortion.

1.3.a. Fetal abnormality.

Anatomical and chromosomal anomalies of the fetus are the most frequently detectable cause of spontaneous abortion. There is an obvious relationship between abnormality of the embryo and its elimination via abortion, but the mechanism by which certain abnormalities lead to abortion is not fully understood. Some abnormal fetuses (e.g. monosomy X, some trisomies and CNS malformations) may survive, whereas others are invariably aborted at an early stage of pregnancy (e.g. autosomal monosomies, polyploidies). The chance of prenatal survival is not necessarily the same as for postnatal survival. Relatively few monosomy-X fetuses are born alive, but once born they usually survive. In contrast, many trisomy-21 fetuses survive until birth, but run a higher risk of early death thereafter.

Most patients who abort spontaneously are counselled on an empirical basis, but the association between fetal abnormality and spontaneous abortion provides a powerful argument for careful morphological and chromosomal analysis of all abortus material. In practice these investigations are infrequently performed, since the cost of karyotyping is considerable and the value of morphology is often underrated, although it is undoubtedly important for the prognosis for subsequent pregnancies.

1.3.a.i. Fetal structural anomalies.

Many classifications of abortus material have been suggested (Mall & Meyer 1921, Fujikara et al 1966, Berry 1980, Rushton 1981). Once more, the under representation of early abortion is a significant cause of discrepancy between reports. Products of conception submitted for examination consist of

variable proportions of fetal, placental and decidual tissue, the proportions being dependant upon the gestational age of the pregnancy, and the way in which the material was obtained. Since most embryos are retained in utero for a time after death, maceration is often marked (Singh & Carr 1967) and most embryos and fetuses are undersized (Brotherton & Craft 1972). The exact frequency of fetuses with major structural abnormalities is difficult to establish because many series have included therapeutic terminations following ultrasound detection of fetal anomalies.

Despite these inconsistencies, some generalisations are possible. The proportion of morphologically abnormal embryos decreases from about 70% between weeks 5 and 8, to less than 10% after 17 weeks. A large proportion of abortions yield incomplete or ruptured sacs, the original contents of which are difficult to assess. Creasy et al (1976) examined 2607 spontaneous abortion specimens. In 804 cases, no identifiable fetus or sac were found. Of the remainder, structural malformations were identified in 4% of singleton and 14% of twin pregnancies. Central nervous system abnormalities contributed 50% of the abnormalities, and several other studies have reported that neural tube defects are the most prevalent morphological abnormality amongst spontaneously aborted fetuses (Nishimura et al 1968, Bell & Gosden 1978, Creasy et al 1976, Fantel et al 1980). A prior history of spontaneous abortion has been associated with an increased incidence of neural tube defects and it has been suggested that remnants of trophoblastic tissue in the uterus may somehow affect the development of the next conceptus (Clarke et al 1975, Gardiner et al 1978). This "trophoblastic rest" hypothesis has not been supported by direct evidence and other authors have postulated that a history of spontaneous abortion may be the manifestation of a tendency towards CNS malformation or other types of disturbed development, rather than a primary cause (Laurence & Roberts 1977, James 1978, Evans 1979).

The incidence of chromosomal abnormalities amongst embryos and fetuses with structural malformations has been reported as higher by some authors (Thiede & Metcalfe 1966, Singh & Carr 1967) and not different by others (Creasy et al 1976, Geisler & Kleinebrecht 1978, Kajii et al 1980). "Blighted ova" have been associated in 50-85% of cases with chromosomal abnormalities (Kajii et al 1980).

The morphological abnormalities observed in pre-implantation and early implantation embryos have been assumed to preclude continued development (Hertig & Rock 1973). However, studies attempting to explain

the 80% implantation failure rate following embryo transfer in IVF patients are now casting doubt on some of these assumptions (Angell et al 1983, Van Blerkom et al 1984, Rudak et al 1984). Several authors have demonstrated that polyploid embryos may exhibit a normal cleavage rate and morphology initially, and can develop through the early implantation phase normally (Mickelman & Mettler 1985). In addition, IVF centres are reporting normal pregnancies after reimplanting morphologically abnormal embryos (Edwards et al 1984). Only in severely fragmented preimplantation embryos has a higher frequency of chromosome anomalies been reported when compared with morphologically healthy embryos (Plachot et al 1987a). Thus an embryo with a cytogenetic abnormality need not exhibit morphological abnormalities, and an embryo that appears morphologically abnormal may develop into a normal child. Although in vitro and in vivo studies may not be comparable, these data suggest that caution is required before correlating the morphological appearance of an early embryo with its likelihood of survival.

Much information can be obtained from detailed examination of the placenta (Rushton 1981). Growth may be impaired from an early stage especially in karyotypically abnormal abortions and many of the abnormalities found in the placenta relate to the time at which embryogenesis is disturbed (Rushton 1978, Honoré et al 1976). The relationship between gestational age and placental weight has been shown to have distinctive patterns for individual chromosomal abnormalities (Phillippe & Boué 1969). Hydropic degeneration, macroscopic cysts and atypical villus stromal cells are seen in 70% of triploid pregnancies and some cases of trisomy (Ornoy et al 1981). In the study reported by Honoré et al (1976) microscopy afforded an 80% accuracy in prediction of chromosomal anomalies.

In a recent study of placental bed morphology (Khong et al 1987) defective haemochorial placentation was noted in association with both sporadic and recurrent abortions, but was not necessarily linked with fetal chromosome abnormalities. Similar defects in placentation are seen in pregnancies complicated by pre-eclampsia and fetal growth retardation (Brosens et al 1972, Khong et al 1986) thereby raising the possibility that all these pregnancy disorders share common morphological features in the placental bed which represent a continuum of pregnancy failure (Redman et al 1984a, Reginald et al 1987).

1.3.a.ii. Chromosomal abnormalities of the fetus.

The first definitive evidence of the relationship between chromosomal abnormality of the fetus and spontaneous abortion was reported by Penrose and Delhanty (1961) who described triploidy in two aborted fetuses. Since then, a large number of studies have established that approximately 50% of all spontaneous abortions are chromosomally abnormal. The introduction of banding techniques (Caspersson et al 1970, Kajii et al 1973) have made possible a more detailed analysis of structural errors and enabled geneticists to find the origin of several numerical and structural abnormalities.

The variation of the reported frequencies of chromosomal anomalies in the major studies ranges from 30-61%. The two principal reasons for this variation are 1) the differences in the methods used to obtain study material and 2) the relative success rates of fetal tissue culture. Since the laboratory is only able to report upon the tissue it receives for analysis, hospital based selection of cases and under-representation of early abortions are potential sources of bias in all studies. The best source of tissue for culture is the fetus itself, but in a high proportion of early abortions cultures have to be initiated from the embryonic placenta or membranes, involving a higher risk of maternal cell contamination. The types of culture (primary or subcultures) and the frequency of successful karyotyping ranged from 36% to 89% in 10 studies reviewed by Lauritsen (1977). Culture failures secondary to non-viable tissue or infective contamination of tissues are both conspicuous problems in the case of "missed abortions" retained in utero for long periods of time.

The frequency of chromosomal anomalies detected in those cases successfully karyotyped is presumed to be representative of all spontaneous abortions, but it is impossible to know the status of those abortuses never received by the laboratory or failing to grow in culture. Culture failures may be disproportionately represented by chromosomal abnormalities since it has been observed that cell cultures from karyotypically abnormal abortuses exhibit slower generation times than normal cells (Cure et al 1974). On the other hand, karyotypically normal fetuses may fail to grow because they were infected in utero. Large series showing very high rates of culture success are less subject to selection bias, but even these studies must be cautiously interpreted. For example, the study of Hassold et al (1980) claims an 89% culture success rate, amongst which 46% of the abortuses were chromosomally abnormal. However, only 60% of the total number of clinically recognised abortions yielded tissue suitable for culture and 53% of the total were

successful. Taken as a percentage of the original number of abortions in the study the frequency of chromosomal abnormalities detected falls to 25%.

A potentially more serious problem is whether the material being cultured truly reflects embryonic status. Discrepancies between embryonic tissue and membranes, in particular mosaicism restricted to the chorion, are being reported in fetal tissues submitted for prenatal diagnosis. This may explain the failure to confirm mosaicism in as many as 30% of cases diagnosed at amniocentesis (Hsu & Perlis 1984, Mikkelsen 1985). Several authors have observed that abnormalities observed in chorion villus samples were not present in cultured embryonic tissue (Simoni et al 1985, Mikkelsen 1987, Crane & Cheung 1988).

Although these findings raise the possibility that the frequency of anomalies amongst abortuses has been overestimated, the inaccuracies involved are probably insufficient to alter the basic belief that about half of all spontaneous abortions are chromosomally abnormal. To add further support to this view the results of the European survey of the incidence of chromosomal abnormalities in spontaneous abortions following IVF treatment has reported the figure to be 62% (Plachot 1989). Furthermore, IVF programmes are now generating human material for research which has provided evidence that 50% of preovulatory oocytes have chromosomal abnormalities (Wramsby et al 1987), and that 30-70% of preimplantation embryos have lethal chromosomal abnormalities (Angell et al 1986, Wimmers & Van der Merwe 1988).

Gestational age and chromosome anomalies.

The fact that gestational age and frequency of chromosomal anomalies share an inverse relationship has already been mentioned (section 1.2.b; p 28) and implies that comparison between studies is only meaningful if they survey the same periods of pregnancy. A 30% incidence of chromosome anomalies was found in the study by Creasy et al (1976) which included only a few cases before 10 weeks and an upper limit of 27 weeks. In three surveys with comparable culture success rates the frequency of chromosome anomalies in clinically recognisable first trimester abortions was reported to be approximately 60%. Menstrual dating was used to classify the abortuses in two of these studies (Kajii et al 1973, Lauritsen 1976) whereas Boué et al (1975a) estimated the gestational age on the basis of a morphological description of the fetus and placenta and in this way selected abortions in which the

development of the fetus was less than 12 weeks. This study had the added advantage of obtaining a high percentage of early abortus tissue, since the participants were all provided with special collection flasks, thereby ensuring that abortions occurring outside the hospital were not lost.

There have been several studies correlating frequency of chromosomal abnormalities with specific gestational intervals, two such studies are summarised in the table below.

Table 1.1.

Prevalence of chromosome abnormality by gestational age .

Week of gestation	Percent chromosomally abnormal abortions	
	Creasy et al 1976	Warburton et al 1980a
4-7	-	14.0
8-11	50.4	49.2
12-15	44.1	39.1
16-19	21.4	18.5
20-27	6.6	11.1

In the study of Warburton et al (1980a), abortions occurring between 4-7 weeks were included and the percentage of chromosomal abnormalities was noted to be lower (14%) than that between 8 and 11 weeks (49.2%). This finding has been reported by other workers (Lauritsen 1976, Takahara et al 1977, Hassold et al 1978) and has led to the suggestion that the low proportion of abnormality detected between 4 and 7 weeks may reflect a tendency for abnormal specimens to be retained in utero. The ultrasound evidence cited earlier of the high frequency of "missed" abortions lends support to this suggestion, but also invalidates future attempts to correlate frequency of chromosomal abnormality with gestational intervals.

Despite these caveats, useful information comes from a recent review of 4 large series reporting the distribution of specific anomalies by gestational interval (Kline & Stein 1987). These authors concluded that each type of chromosomal abnormality has a particular pattern of intra-uterine survival, and that the time of abortion for chromosomally abnormal conceptions is determined primarily by the type of abnormality. Both monosomy X and trisomic abortions exhibit modal distributions, the majority of abortions occurring between 11 and 14 weeks gestation, whereas triploid abortions show

a more uniform distribution throughout the gestational age range 7 to 18 weeks. Furthermore, a particular chromosome abnormality tends towards a certain type of morphological development. For example, intact empty sac in trisomy 16, presence of a cord without recognisable embryo for monosomy X, an anatomically organised embryo or fetus for trisomy 21. Hence, in a chromosomally abnormal conception, the abnormality is the major determinant of the degree of morphological development and of the probability of fetal death at different stages in gestation.

1.3.a.iii. Types of chromosomal abnormality.

Autosomal trisomies.

Autosomal trisomies account for over 50% of all the abnormal chromosome complements detected in spontaneous abortions. One-third of all trisomies involve chromosome 16, and other frequently noted trisomies include numbers 22(10%), 21(8%), 15(7%), 13(6%), 2(6%), and 18(5%), the remainder being distributed in gradually declining frequencies over the other chromosomes (reviewed by Chandley 1981). All except trisomy 16 are observed in liveborns, but the relative frequencies differ among abortuses and liveborns. The mean pregnancy duration for trisomic fetuses has been estimated at 8 weeks (Boué et al 1976) but trisomies 1,5,9 and 11 are rarely detected in abortuses and are thought to result in very early developmental arrest and unrecognised abortion (Creasy et al 1976). The overall increase in chromosomal abnormalities observed with rising maternal age is almost entirely due to the increased frequency of trisomic fetuses (Boué et al 1975, Takahara et al 1977, Kajii et al 1980). This age effect is usually ascribed to disturbed disjunction amongst older women, but interestingly the maternal age effect is not pronounced in trisomy 16.

Monosomy.

Monosomy is the single most common abnormality in spontaneous abortions, accounting for 20-25% of abnormal specimens. The mean pregnancy duration is usually about 6 weeks (Boué et al 1976) and since the birth incidence of Turner's syndrome is estimated as 1 in 20,000 newborns, the survival ratio of affected fetuses is probably about 1 in 300 (Kajii et al 1980). Embryonic losses may consist of only an umbilical cord stump, whereas at later gestations characteristic "Turner" stigmata (horseshoe kidney, cystic hygromata and generalised oedema) are seen.

Monosomy X is also associated with a decrease in mean maternal age compared to chromosomally normal abortions and term births (Kajii & Ohama 1979, Warburton et al 1980b). Monosomy-Y is supposedly lethal because it has never been found in abortuses or liveborns.

Polyploidies.

Polyploidy results from additional haploid chromosome complements and accounts for 15-20% of all chromosomal abnormalities. The incidence is not affected by maternal age. The chromosome complements are usually 69XXY or 69XXX, whereas 69XYY is rare. The mean duration of survival is about 5 weeks of embryonic life. Although there is a large variation in developmental age among triploid abortuses, they rarely survive to term. Triploid abortuses show a very variable morphology ranging from empty sacs to embryos with major anomalies and large fetuses. The malformations consist primarily of neural tube defects and omphaloceles and characteristic placental hydropic degeneration which becomes more pronounced with increasing length of gestation. Partial hydatidiform moles are invariably triploid, but in contrast to moles, hydropic degeneration is hypoplastic in triploid conceptions.

Tetraploid conceptions are uncommon accounting for approximately 5% of all recorded abnormalities. The abnormality appears to cause developmental arrest very early, the majority of specimens being empty sacs or grossly abnormal embryos less than 5mm long.

Structural rearrangements.

Structural chromosome rearrangements account for 3-5% of chromosomally abnormal abortuses. They may arise de novo during gametogenesis, or be inherited from a parent carrying a balanced translocation or an inversion. Structural rearrangements are a relatively more common explanation for repetitive abortions (Simpson 1986) and are discussed further below (1.3.a.vii; p 34-38).

Other abnormalities.

Sex chromosome polysomies are observed infrequently in abortus specimens and few data exist with respect to morphological or histological findings. Some of them survive as individuals with Klinefelter syndrome (47XXY) or as phenotypically normal males (47XYY) or females (46XXX). Mosaics are found infrequently in spontaneous abortions (Kajii et al 1980) but may arise in vitro during cell culture and are being reported more recently in chorionic tissue examined for abnormality in pre-natal diagnostic procedures.

1.3.a.iv. Origin of chromosomal abnormalities.

With the exception of a small proportion of inherited translocations nearly all the abnormalities seen in abortuses arise *de novo* either during meiosis (trisomy, monosomy, triploidy), during fertilisation (triploidy) or during the first cleavage division (tetraploidy). Mosaics arise during an abnormal mitotic division sometime after fertilisation. Many of these are trisomic zygotes in which the normal cell line arises mitotically.

Studies of regions of certain chromosomes which show variation between individuals can identify the origin of the extra chromosome in informative cases. Most trisomies (80%) arise from maternal non-disjunction (Jacobs & Hassold 1980) although paternal non-disjunction has been reported (Lauritsen 1977). Similar studies in livebirths have shown that 79% of cases of Trisomy 21 are maternal in origin (Mikkelsen 1982). The vast majority of these errors occur in the first meiotic division (Boué et al 1985, Jacobs & Hassold 1980).

Similar studies of triploids have shown that 73% have two paternal sets of chromosomes, and the majority of these result from dispermy. The remaining 37% are derived from diploid oocytes (Boué et al 1985). Tetraploids arise during the first mitotic division after fertilisation when cytoplasmic cleavage fails to occur (Boué & Boué 1977, Kajii & Niikawa 1977). It is not possible to demonstrate the origin of the X chromosome in monosomy X in abortuses by these methods and hence we cannot compare abortuses with survivors, in whom 75% have a maternal X chromosome. The hypothesis that monosomy X results from the loss of the paternal sex chromosome at early cleavage (Chandley 1981), should be possible to test using restriction fragment length polymorphisms (Creasy 1988).

1.3.a.v. Sex ratio among spontaneous abortions.

The sex ratio determined by karyotyping (defined as the ratio between the number of abortuses with a Y chromosome and those lacking this chromosome) has been estimated in three large surveys (Carr 1967, Boué & Boué 1973b, Lauritsen 1976) and did not differ significantly from unity. It is now generally accepted that 45X embryos should be excluded from the figures and that the presence of a Y chromosome in polyploidies should indicate male sex. The inadvertant culture of maternal cells may increase the proportion of "female" conceptuses and explains the apparent increase of females in early

abortus material. Late abortions (after the 20th week) have a slight excess of males, similar to that seen among stillbirths and infants at term (Creasy 1988).

Chromosomal abnormalities of the fetus affect the sex ratio at birth. Most studies of trisomic abortuses have found a preponderance of males, although two of the largest studies have recorded an excess of females. One of these included a large proportion of early abortuses (Boué et al 1975) while the other contained large numbers of late abortions and found an excess of males in the chromosomally normal abortions (Creasy et al 1976). There may be differences in the sex ratio between different trisomies. Trisomies 21 and 18 both show an excess of males in abortuses (sex ratios 1.7 and 1.8 respectively) although at term trisomy 21 shows a slight male preponderance whilst 80% of liveborn infants with trisomy 18 are female. Trisomy 9 shows a marked excess of females in abortuses, whereas in trisomy 16 there is a small increase in males (Hassold et al 1980).

1.3.a.vi. Factors predisposing to chromosomal abnormalities.

Although developments in cytogenetic techniques have revealed much information about types of chromosomal aberration associated with abortion, the mutagenic factors which cause chromosome anomalies in the fetus are largely unknown. The abnormalities observed may be the result of a complex interaction between genetic and environmental factors.

Individual genes causing non-disjunction may account for some chromosomal anomalies seen in spontaneous abortions. Single mutant gene disorders and those disorders inherited in polygenic fashion are four times as frequent at birth than chromosomal abnormalities (Simpson 1980) It may be that only part of the selection procedure which appears to "screen out" chromosomal errors during development is operative in these disorders, and that a proportion of the 50% of abortions which are not chromosomally abnormal are due to other genetic errors. Indeed, this appears to be the case for multifactorial disorders such as neural tube defects. The advances in molecular genetics and their application to the identification of genetic markers in both parents and their offspring promises a better understanding of these disorders.

Maternal age and its effect on the frequency of chromosomal abnormalities has already been discussed. Age changes occurring in ova and spermatozoa may result in an increased frequency of malformation, but data in humans is sparse. Ageing in oocytes may be coincident with maternal

ageing, over-ripening in the follicle (delayed ovulation) or postovulatory (delayed fertilisation). There is a suggestion that delayed ovulation may lead to polyploidy (Boué et al 1975a, Salisbury et al 1977) and that both delayed fertilisation and spermatozoal ageing within the female genital tract is associated with spontaneous abortion (Guerrero & Rojas 1975).

Three environmental factors have been studied in relation to chromosomal anomalies and spontaneous abortions, oral contraceptives, irradiation and cigarette smoking and are discussed in a later section (4.) The importance of recurrent aneuploidy, parental translocations, inversions and other chromosomal variants in predicting the risk of recurrent pregnancy loss is discussed below.

In summary, the contribution of chromosomal disorders to embryonic and fetal loss is now well documented in humans. In livebirths the incidence is 0.6% (Nielsen 1975) a 10-fold increase is found in stillbirths (Machin & Crolla 1974) and a 100-fold increase is reported in spontaneous abortions (Boué & Boué 1976).

1.3.a.vii. Recurrence risk of chromosomal abnormalities.

In addition to the contribution that chromosomal abnormalities of the fetus make to sporadic spontaneous abortion, they are an important cause of recurrent abortion. Familial recurrence of chromosomal abnormalities are a well recognised phenomenon (Verp & Simpson 1985). They may be due to recurrent aneuploidy or parental chromosome abnormalities.

Recurrent aneuploidy.

Follow-up studies of the parents of karyotyped abortions have shown that a couple's risk of subsequent abortion correlates with the number of previous abortions and with the karyotype of the abortions. Couples who have a chromosomally normal abortion have had more previous abortions and fetal losses (Geisler & Kleinebrecht 1978, Hassold et al 1980) and run a higher risk of having another abortion than couples having an abortion with abnormal karyotype (Lauritsen 1976). In couples with no history of abortion other than the karyotyped one, the frequency of abortion in the subsequent pregnancy is about 15%, with a higher frequency in the karyotypically normal cases compared to the karyotypically abnormal cases (Boué et al 1975a, Lauritsen 1976). If there has been an abortion previous to the karyotyped abortion the recurrence risk of abortion for those couples with a karyotypically normal abortion is approximately 40% whereas in couples in whom the index

abortion is karyotypically abnormal the risk of subsequent abortion is significantly smaller averaging 21% (Boué et al 1975a, Lauritsen 1976). These observations suggest that chromosomally normal abortions are less random than abnormal ones (Hassold 1980).

Studies in which two consecutive abortuses have been karyotyped have shown a trend towards both abortuses being karyotypically normal or both karyotypically abnormal (Kajii & Ferrier 1978, Boué & Boué 1973a, Alberman et al 1975, Lauritsen 1976). If the complement of the first abortus is normal the chance of the second abortus also being normal is 70%. Conversely, if the first abortus is chromosomally abnormal the risk of a further chromosomally abnormal abortion is 80% (Simpson & Bombard 1987) but the type of abnormality need not be the same (Boué & Boué 1973a).

The most common recurrent aneuploidy is trisomy (Simpson 1980, Warburton et al 1980a) but it may well involve another chromosome (Hassold et al 1980, Boué et al 1975). Women who have trisomic abortuses also have a higher incidence of trisomic births in their previous pregnancies (Alberman 1981, Warburton et al 1982). For example, the frequency of Down's syndrome among viable siblings of trisomic abortuses is 10 times higher than expected (Alberman et al 1975). This implies that not only does trisomy seem to recur in certain couples, but also that some couples are at greater risk of conceiving successive fetuses which are chromosomally abnormal, some of which are lethal and are aborted, whereas others survive. Part of this effect may result from maternal age, but this seems unlikely to explain the entire phenomenon since the increased risk of a second trisomic liveborn is greatest when the mother is relatively young at the time of her first trisomic infant being born (Stene et al 1984).

The clinical significance of these observations is considerable. Some couples have an increased risk of abortion because they produce chromosomally abnormal gametes, not as a result of translocation but presumably because of an increased tendency toward non-disjunction, possibly inherited and possibly environmentally induced. Identification of these individuals would permit antenatal diagnosis in subsequent pregnancies and facilitate our understanding of the causes of non-disjunction. Conversely, among those couples whose abortuses are chromosomally normal, one would search for either nongenetic, Mendelian or polygenic causes.

Translocations.

Studies examining the effect of parental chromosome abnormalities upon the incidence of spontaneous abortion have found there to be a significant association in couples experiencing recurrent spontaneous abortions, but the prevalence of abnormality in sporadic cases to be very low. Thus in one survey, the frequency of chromosomal abnormalities in one of the parents of a single spontaneous abortion was 0.76% (Lauritsen 1976) whereas in 7.2% of couples with recurrent episodes of abortion a structural chromosomal abnormality was present in one of the partners, which is twenty times the rate in the newborn population (Boué et al 1985).

The recurrence risk of abortion is dependant on the type of abnormality and may be as high as 38% for couples in whom one partner is a reciprocal translocation carrier (Lindenbaum & Bobrow 1975). Approximately 1 in every 600 individuals in the general population is a balanced translocation carrier. "Translocation" describes the situation in which a fragment of one chromosome becomes attached to the broken end of another. A reciprocal translocation involves an exchange of material between 2 non-homologous chromosomes. Robertsonian translocations are limited to the acrocentric chromosomes (13,14,15,21,22) and require loss of the short arms from each of two chromosomes followed by fusion of their long arms at the centromeres. One of the resulting chromosomes is extremely small and is lost in subsequent mitotic divisions. Although individuals with balanced translocations are phenotypically normal, their gametes may show chromosomal duplications or deficiencies as a result of normal meiotic segregation. The zygote may be normal, a balanced translocation carrier like its parent, trisomic for part of a chromosome, or monosomic for part of a chromosome. The last two situations almost always lead to abortion.

The frequency of balanced translocations in the general population has been estimated as 0.2 - 0.3% (Jacobs et al 1977, Hamerton et al 1975, Nielsen & Sillesen 1975). There are numerous studies of the frequency of balanced translocations and other chromosomal abnormalities and variants in couples with recurrent abortion (Kim et al 1975, Schmidt et al 1976, Mennutti et al 1978, Fitzsimmons et al 1983, Schwartz & Palmer 1983, Sachs et al 1985, Campana et al 1986, Adamoli et al 1986). The interpretation of these studies is not straightforward, because of the many variables influencing the results. For example, balanced translocation has a strong association with congenital defects other than abortions, banding techniques have improved translocation

detection, and the populations of patients studied vary markedly. These caveats help to explain why in one series of 100 couples with two or more consecutive abortions but no congenitally abnormal offspring, no balanced translocations were found (Ward et al 1980) whereas in two other studies the prevalence in couples with a history heavily weighted with congenital defects, the percentages were 27.3% (Byrd et al 1977) and 31.2% (Stenchever et al 1977).

Balanced translocations are usually detected when cytogenetic studies are performed in couples who have repeated abortions or a liveborn child with a congenital malformation. Overall, the frequency of balanced translocations in couples with a history of two or more abortions is about 3-5% (Simpson et al 1981). In a review of the literature which included over 1300 couples, Davis et al (1982) concluded that the rate of balanced translocation in couples with a history of abortions and stillbirths only was 3.5%. If couples with normal children were included the probability was 4.2% and when histories included abortion and fetal malformations the figure averaged 9.2%. A positive relationship between the frequency of translocation carriers and the number of previous abortions has been noted in a few studies (Husslein et al 1982, Schwartz & Palmer 1983, Campana et al 1986) but no demonstrable trend was found in others (Michels et al 1982, Sant-Cassia & Cook 1981, Turleau et al 1979, Sachs et al 1985). Couples whose abortions have all occurred in the first trimester of pregnancy have been shown to have an increased frequency of balanced translocations when compared to couples with second trimester losses (Schwartz & Palmer 1983). Women are twice as likely as men to show a translocation, which has been attributed to the fact that structural abnormalities of chromosomes in males may be associated with sterility (Lippman-Hand & Vekemans 1983, Simpson & Bombard 1987, Campana et al 1986).

If chromosomal variants are included, the incidence of couples with repeated abortions and chromosomal abnormalities increases to 5-10% (Simpson 1980, Blumberg et al 1982, Schwartz & Palmer 1983, Campana et al 1986). Pericentric inversions occur in about 1% of the population and are usually considered to be a normal variant, although crossing over and recombination within a pericentric inversion loop may produce unbalanced gametes (Simpson et al 1981). The frequency of inversions found among couples with recurrent abortion varies from normal (Simpson et al 1981, Husslein et al 1982) to 4-5% (Mennutti et al 1978, Tibiletti et al 1981) and has a predelection for chromosome 9 (Campana et al 1986).

An association between maternal sex chromosome mosaicism and recurrent abortion has been reported (Hsu et al 1972, Hecht 1982, Andrews & Roberts 1982, Holzgreve et al 1984, Sachs et al 1985). The prevalence of abortion in women with a 45 X cell line may be as high as 50% (Singh et al 1980, Reyes et al 1976). The presence of large satellites on acrocentric chromosomes is generally considered to be of no clinical significance. However, mothers of boys with a long Y-chromosome appear to have significantly more abortions than those with sons carrying a Y-chromosome of normal length (Patil & Lubs 1977, Nielsen 1978) and the Y-chromosome of the male partner of recurrently aborting couples is either too short or too long when compared to controls with live children (Verp et al 1983).

1.3.a.viii. Patient counselling.

These data emphasise the importance of parental karyotyping of couples with repeated abortions. Financial restrictions usually dictate that cytogenetic studies are only initiated after 3 pregnancy losses although the incidence of balanced translocations is increased significantly over the general population in couples with a history of two abortions. Couples with one normal live child in addition to their spontaneous abortions are at increased risk also. If a balanced translocation is found genetic counselling may provide the couple with information regarding the risk of another abortion and in a subsequent ongoing pregnancy pre-natal diagnosis in the form of amniocentesis or chorion villus sampling should be performed to identify an unbalanced translocation in the fetus.

Recurrence risks vary in different Robertsonian translocations. In the most common translocation involving chromosomes 14 and 21, one-third of the viable offspring of balanced carriers should theoretically have Trisomy 21 and 50% of all gametes should be lethal. However, empirical data from amniocentesis registries reveal that only 10-15% of the viable offspring have Trisomy 21 (Boué & Gallano 1984), and the data are similar for liveborn infants. Far more abnormal offspring are found when chorion villus sampling is used since the diagnosis is made so much earlier in gestation (Mikkelsen 1985). If a Robertsonian translocation involves homologous chromosomes the prognosis is hopeless. The only liveborns are abnormal (Trisomy 13 or 21) and all other conceptions terminate in spontaneous abortion. Couples with this type of translocation should be informed about embryo transfer techniques, artificial insemination or sterilisation.

Amniocentesis surveillance of women and men with balanced reciprocal translocations demonstrates that their risk of an unbalanced fetus is 12% (Boué & Gallano 1984). Although substantial, these risks are far less than the theoretical risk of 50%, since natural selection via early abortion occurs, unbalanced products are recovered from 40% of chorionic villus samples (Mikkelsen 1985). Phenotypically normal offspring are equally likely to inherit normal chromosomes or the parental balanced translocation. In the case of chromosomal inversions, the empirical risk of a fetal abnormality is 8% for females and 4% for males, (the difference being consistent with the higher frequencies of recombination in females (Weitkamp et al 1973) which is significantly lower than the theoretical risk of 50%.

Wherever possible, cytogenetic analysis of abortus material from couples with repeated abortion should be performed irrespective of the parental karyotype, since the findings may influence the advice offered to the couple for management of a future pregnancy, as mentioned above in the section describing recurrent aneuploidy (Alberman 1981, Kohn et al 1976).

1.3.b. Anatomical.

1.3.b.i. Congenital abnormalities of the uterus.

The incidence of uterine anomalies in the general population is unknown, estimates ranging from 1 in 700 women (Glass & Golbus 1978) to 1 in 30 (Greiss & Mauzy 1961) and 1 in 10 pregnant women (Hay 1958). Comparisons between reports are difficult since the classification of the different types of anomalies and the methods used for their diagnosis are variable. Most importantly, the study populations from which the figures are derived are usually small, reflect the interests of the clinician (Green & Harris 1976) and are seldom representative of the normal female population, being more usually based on patients with a history of infertility or recurrent late reproductive failures. In a review of the available data Stoot (1978) concluded that the incidence of all congenital uterine anomalies varied from 0.1% to 10% of patients investigated and that the prevalence in the general female population could be estimated at about 1%. Of the subtypes of uterine anomaly, bicornuate uterus is diagnosed most frequently (50%), followed by arcuate uteri (20%) and septate and subseptate uteri (10%). Relatively rare are uterus didelphys and unicornuate uterus.

Although uterine anomalies may account for about 12% of all cases of recurrent abortion (reviewed by Bennett 1987), most authors conclude that

only a minority of women with uterine anomalies experience reproductive difficulties (Glass & Golbus 1978, Bennett 1987). Hence, the majority of congenital uterine anomalies will remain unrecognised, the majority of women with recognised anomalies will have successful pregnancies and women in whom the diagnosis of both congenital anomaly and pregnancy wastage have been made can achieve successful pregnancies without any treatment (Jones & Jones 1953, Jones & Wheelless 1969).

An incidence of spontaneous abortion of 30% has been reported by several authors (Hay 1958, Stoot 1978, Heinönen 1982), suggesting that the risk of abortion for these patients with congenital uterine anomalies is approximately double that of the normal population. When attempting to assess the risk for a particular individual, other factors such as parity, past obstetric history and the type of anomaly need to be taken into account. Historically, uterine anomalies were associated primarily with late second trimester pregnancy losses. More recent reports have demonstrated that uterine anomalies increase the risk of spontaneous abortion in the first trimester of pregnancy (Rock & Zacur 1983, Buttram & Gibbons 1979, Musich & Behrman 1978). In one study the incidence of uterine anomalies amongst patients complaining of recurrent first trimester losses was 12% compared to a 7% loss rate in patients with recurrent second trimester abortions (Stray Pedersen 1984). Moreover, the incidence of threatened first trimester abortion in pregnancies continuing to term successfully is reported to be as high as 33% (Heinönen 1982).

Early spontaneous abortions have been most frequently associated with bicornuate and septate uteri in which the relatively avascular septum is presumed to be the site of implantation, thereby leading to an early pregnancy failure. However, double uterus (Jones 1957) and unicornuate uterus (Buttram and Gibbons 1979) have also been associated with early losses, although these abnormalities are more frequently associated with second trimester abortion. Cervical incompetence is associated with congenital fundal anomalies in 30% of cases (Craig 1974, Bennett 1987).

The diagnosis of uterine congenital anomalies relies heavily on the clinician's index of suspicion. The presence of a uterine septum can be sought at the time of evacuation of retained products of conception. Hystero-graphy (HSG) or hysteroscopy should be performed if an abnormality is suspected, together with laparoscopy to inspect the external aspect of the uterus and adnexae. The use of ultrasound in experienced hands is a useful non-invasive

screening procedure for congenital abnormalities. In addition it can be used to examine the renal tract, since there is an association between uterine and renal anomalies in 9% of patients (Rock & Zacur 1983) which is of particular importance when surgical correction is being considered.

Remarkable pregnancy success rates have been reported following metroplasty, the surgical reconstruction of the bicornuate or septate uterus. Strassman (1966) reported a fetal salvage rate of 86% and Craig (1974) reported a 94% pregnancy success rate using the Tompkins metroplasty (1962). In a review of 11 treatment series (Stoot 1978) the percentage of liveborn infants was between 80% and 90%. However, metroplasty is complicated by pelvic adhesion formation and a significant number of women never conceive again after this type of surgery. In 2 independent reviews of the available literature (Huisjes 1984, Bennett 1987) the incidence of subsequent infertility averaged 30%, which seems a high price to pay for a treatment mode which has never been compared in a randomised controlled trial.

The use of an operating hysteroscope to resect the septum has been reported, with encouraging results (Israel & March 1984, DeCherney & Polan 1983, Daly et al 1983). This procedure involves simultaneous laparoscopy, is performed in the follicular phase of the menstrual cycle and is followed by 4-8 weeks of oral oestrogen therapy. The advantages of this technique are that major surgery is avoided, hospital stay may be as short as 24 hours, complications reported to date are few and subsequent pregnancies do not require delivery by Caesarean section. If further studies confirm that fertility is not compromised significantly, hysteroscopic metroplasty may be expected to become the procedure of choice for the septate uterus (Bennett 1987).

1.3.b.ii. DES exposure.

The use of the non-steroidal oestrogen diethylstilboestrol (DES) in the management of many pregnancy complications including threatened abortion, recurrent abortion, premature labour and stillbirth became commonplace in the 1950's and 1960's following the reports of its efficacy by Smith (1948). Although the prospective study of Dieckmann et al (1953) failed to find any benefits in patients receiving DES compared to a control group, an estimated three million women were treated with DES during their pregnancies. The drug was withdrawn from the market in 1971 following reports of the association of vaginal clear cell adenocarcinoma with in utero exposure to DES (Herbst & Scully 1970).

The first evidence that in utero DES exposure was associated with upper genital tract abnormalities in addition to the risk of malignancy, emerged in 1977 (Kaufmann et al) and was followed by several reports in which over 60% of DES exposed female fetuses were found to have uterine abnormalities diagnosed by hysterosalpingography (Kaufmann et al 1980, Rosenfeld & Bronson 1980, Ben-Baruch et al 1981). The abnormalities observed most frequently were a T-shaped uterus, uterine cavity constriction bands, hypoplasia and widened lower segment. In addition, intra-uterine filling defects were recorded in approximately 35% of patients, the most common being irregular margins, synechiae and polypoid lesions. A significant relationship exists between the occurrence of vaginal epithelial changes, structural abnormalities of the cervix and the presence of upper genital tract abnormalities. According to Kaufmann et al (1980) the finding of a lower genital tract lesion should alert the clinician to the increased likelihood of an abnormal hysterosalpingogram (HSG). However the incidence of similar HSG abnormalities in a control population of non DES exposed women is not known and reports of T-shaped uterine cavities antedating the use of DES have been made (reviewed by Barnes 1980).

The adverse effects of these uterine changes on pregnancy outcome have been variably reported. Although Cousins et al (1980) found no significant increase in the number of early spontaneous abortions, the majority of reports have concluded that the risk of spontaneous abortion is increased after DES exposure (Berger & Goldstein 1980, Kaufman et al 1980, Rosenfeld & Bronson 1980, Sandberg 1981, Veridiano et al 1981, Mangan et al 1982). The incidence of spontaneous abortion in DES exposed patients enrolled in the National Cooperative Diethylstilboestrol Adenosis Project was 26% compared to only 16% in a control group (Barnes et al 1980). In a follow up study of the daughters of women who had taken part in a placebo controlled trial of the effect of DES on abortion many years previously, Herbst et al (1981) found that the spontaneous abortion rate in the first pregnancies of DES exposed daughters was 21% compared to 11% in placebo daughters. In a later review of the literature by Kaufman (1987) an average incidence of spontaneous abortion of 24% amongst all evaluable pregnancies was calculated from 8 studies. In the largest study of 676 DES exposed women poor pregnancy outcome was correlated with the HSG findings in 327 women, but it is recommended that HSG examination should be limited to those DES exposed women who have experienced an unfavourable pregnancy outcome

in the past or who have obvious lower genital tract abnormalities. The results of cervical cerclage treatment for this group of women has not been shown to significantly improve their pregnancy outcome (Kaufman et al 1984).

1.3.b.iii. Intra uterine adhesions.

Intra-uterine adhesions (or synechiae) were first described by Fritsch (1894) but the condition is universally referred to as Asherman's syndrome (1948). In the vast majority of cases pregnancy is the predisposing factor (Schenker & Margiolath 1982). Most cases follow vigorous uterine curettage in the post abortion or post-partum period, when the maternal hypo-oestrogenic state may result in poor endometrial proliferation and predisposes the patient to the formation of intra-uterine adhesions (Buttram & Turati 1977). Intra-uterine adhesions studies are estimated to be present in 1.5% of all patients who undergo hysterosalpingography (HSG) (Dmowski & Greenblatt 1968), 15% of patients after curettage for spontaneous first trimester abortion (Adoni et al 1982) and 68% of women with infertility following two or more uterine curettages (Asherman 1950).

Intra-uterine synechiae have been implicated in both sporadic and recurrent abortion. Recurrent abortion was noted in 14% of 1973 patients with uterine adhesions reviewed by Schenker & Margiolath (1982). The abortions may occur because of the reduced dimensions of the uterine cavity or the lack of sufficient endometrium suitable to support the growth of the fetus. Polishuk et al (1977) suggested that repeated pregnancy losses were due to defective endometrial vascularisation caused by damaged uterine arterial vessels responding poorly to oestrogen stimulus.

Several classification systems have been suggested to categorise the severity of adhesion formation based on HSG findings (Buttram 1977, Toaff & Ballas 1978) and the type, extent and site of uterine adhesions as viewed by hysteroscopy (March et al 1981, Weseley 1981). Although useful as a standard method of reporting results and thereby allowing comparison of treatment regimes, no correlation between the extent of adhesion formation and pregnancy outcome following therapy has been demonstrated (Bergquist et al 1981). The treatment protocols most frequently used include dilatation and curettage or lysis of the adhesions via a hysteroscope, followed by insertion of an intra-uterine contraceptive device for up to 2 months or a foley catheter for 7-10 days. Although successful pregnancies after hysteroscopic lysis alone have been reported (March & Israel 1981), the majority of studies conclude that the

addition of prophylactic post-operative antibiotics, cyclic oestrogen and progesterone supplementation are required to significantly reduce the incidence of spontaneous abortion (Jewelewicz et al 1976, Rock & Zacur 1983). In the series of Bergquist et al (1981) the spontaneous abortion rate improved from 78% to 20% with combined therapy and the successful pregnancy rate for recurrent aborters was 52% using this regimen. The meta-analysis review conducted by Jansen (1982a) concluded that the addition of adjuvant oestrogen therapy reduced the incidence of spontaneous abortion significantly from 35% to 7.5%.

1.3.b.iv. Retroverted uterus.

It is questionable whether uterine retroversion increases the risk of spontaneous abortion, but the possibility is based on a study by Tupper et al (1957), who found that retroversion occurred twice as often in patients who miscarried than among those who carried their pregnancy to term. Two more recent studies (Weekes et al 1976, Franke 1973) have found increased frequencies of abortion in women with retroversion, but the studies were not controlled for other variables known to influence the abortion rate. Huisjes (1984) suggests that 10% of all Caucasian women have uterine retroversion during the first trimester of pregnancy and that the finding should be considered as a normal variant without pathological consequences.

1.3.b.v. Leiomyomas.

It is possible that uterine leiomyomata, depending on their size and location may interfere with implantation and early pregnancy development (Malone & Ingersoll 1975). Blood flow studies have revealed decreased flow rates in myomas and adjacent tissues when compared with the normal uterus (Forssman 1976), and various endometrial abnormalities ranging from atrophy to hyperplasia have been associated with myomas (Deligdish 1970).

Little data is available concerning the relationship between this common gynaecological problem and reproductive outcome. In a review of 1,941 cases of myomectomy in which preoperative and postoperative abortion rates were recorded, Buttram & Reiter (1981) noted that the spontaneous abortion rate was reduced from 41% to 19% after myomectomy, but no control group was included. In another study (Babaknia et al 1978) a successful pregnancy rate of 50% was reported for women with recurrent pregnancy losses undergoing myomectomy, but again this data is uncontrolled. Since the

association between leiomyomas and recurrent abortion is tenuous, it has been suggested that myomectomy should be considered as a treatment option for women with recurrent abortion only when all other possible aetiological factors have been eliminated (Rock & Zacur 1983).

1.3.b.vi. Cervical incompetence.

The true incidence of cervical incompetence is unknown, but the condition is believed to be strongly associated with recurrent spontaneous abortion. However, since a history of second trimester pregnancy loss is one of the diagnostic criteria (WHO 1970), an incompetent cervix is rarely diagnosed in women who have not had pregnancy losses or have not been pregnant. In a population of women seeking prenatal care the frequency was estimated to be 3.5 per 1000 (Niswander & Gordon 1972). This figure is increased when the women studied have been recruited following one spontaneous abortion (Miller & Poland 1973) and greatly increased when the population studied consists of women with a history of multiple abortions (Tho et al 1979, Strobino et al 1986).

The reported incidence of spontaneous abortions due to an incompetent cervix ranges from 1 -18 per 1000 deliveries (Lipshitz 1975, Toaff et al 1977). The incidence is thought to be increased after therapeutic termination of pregnancy by some authors (Ratten & Beischer 1979, Wesley 1981) but not by others (Furness 1983, Daling & Emmanuel 1977). However, most studies conclude that cervical incompetence is an important cause of second trimester abortion and may account for one in five cases of late abortion (Mann et al 1961, Stromme & Haywa 1963, McDonald 1980).

Incompetence of the cervix is due to a weakness in the sphincter of the internal os, which allows the contents of the pregnant uterus to extrude when they reach a critical weight near the 20th week of pregnancy. The weakness may be congenital, but is more usually acquired following dilatation and curettage, cervical amputation or cone biopsy or lacerations at the time of a previous delivery. In the series of 269 cases reported by McDonald (1980) less than 2% of the cases occurred in non-parous women who demonstrated no aetiological factors. Several authors have drawn attention to the association between cervical incompetence and DES exposure (Goldstein 1978, Singer & Hochman 1978, Quinlan & Cruz 1980, Cousins et al 1980). Familial occurrence of cervical incompetence has also been described (Ranney 1963, Jennings 1972).

The diagnosis is difficult to establish objectively but is usually based on a history of rapid, painless mid-trimester abortion or pre-term delivery. In the non-pregnant state the passage of a Hegar 8 dilator without resistance during the post-ovulatory phase of the menstrual cycle, or the finding of a wide or funnel shaped uterus in pre-menstrual hystero-graphy may be used to diagnose cervical incompetence (Mann et al 1961, Anthony et al 1982). In early pregnancy ultrasound examination of the cervix may be helpful (Brook et al 1981, Sarti et al 1979) and in addition will help to exclude other causes of midtrimester abortion such as severe congenital fetal abnormalities and uterine malformations.

The surgical procedures described for the treatment of cervical incompetence share the same basic principle, the insertion of an encircling ligature around the cervix at the level of the internal cervical os to produce its total or partial occlusion. The Shirodkar cerclage (1955) involving upward reflection of the bladder base is usually performed prior to pregnancy. The McDonald suture (1957) involves a simple cerclage of the cervix and is usually performed in the second trimester of pregnancy

Many uncontrolled studies have reported the benefit of cerclage in terms of the rate of abortion before and after treatment in the same women (Seppälä & Vara 1970, Toaff et al 1977, McDonald 1980, Harger 1983, Crombleholme et al 1983, Schwartz et al 1984, McDonald 1987). These results suggest that successful pregnancy outcomes between 80 and 90% can be expected when the suture is inserted prophylactically. In a review of 250 cerclage procedures by Harger (1980), the fetal survival rate after elective Shirodkar suture (87%) or McDonald suture (78%) was not significantly different. Since the McDonald technique is much simpler and equally effective the Shirodkar technique is now less frequently used.

The lack of randomised prospective studies have cast doubt upon the efficacy of cervical cerclage treatment for cases of recurrent mid-trimester abortion due to cervical incompetence. Such studies have been difficult to organise since the treatment is already widely practised empirically, making the inclusion of valid control subjects a difficult ethical consideration (Editorial Lancet 1977). Possibly the increased scrutiny of patients with a cervical suture in situ has an effect on successful outcome (Vere 1982), and Crombleholme et al (1983) concluded that cervical cerclage should be considered in the management of recurrent abortion even when there was no typical history of cervical incompetence. Conversely, Harger (1983) and Tho et

al (1979) argue that the 70% spontaneous resolution rate in idiopathic recurrent abortion makes the use of cerclage unwarranted in the absence of an untreated control group.

The results of a prospective trial by Rush et al (1984) suggested that cervical cerclage did not prolong gestation or improve fetal survival. Those patients with a suture spent significantly longer periods in hospital during the remainder of their pregnancy, were more likely to receive tocolytic drugs and experienced a higher incidence of puerperal pyrexias. The definitive results from the Medical Research Council/Royal College of Obstetricians and Gynaecologists multicentre randomised trial of cervical cerclage are still awaited. This ongoing study is open to those patients whose obstetrician is uncertain about the advisability of cerclage. Patients for whom cerclage is considered definitely advisable or inadvisable have not been included. A preliminary report of the trial has been published (1988) presenting the results of the interim analysis after recruitment of 900 patients. The results suggest a beneficial effect of cerclage upon length of gestation and vital outcome. There were 5% fewer deliveries between 20 and 32 weeks gestation which is equivalent to the prevention of one pre-term delivery for every 20 sutures inserted. However this result is of marginal statistical significance and uncertainty remains as to whether any real benefit has yet been demonstrated.

1.3.c. Infective.

A variety of organisms including bacteria, viruses, mycoplasmas and parasites have been associated with pregnancy loss, but proof of a causal relationship between abortion and a specific infectious agent is difficult to establish. Superimposed bacterial infection may occur after fetal death has occurred. Culturing the uterine contents may yield unreliable results, because obtaining material uncontaminated by cervical and vaginal flora is difficult. Over half of the products of abortion appear to be infected, mostly with organisms normally present in the cervix (Bartizal et al 1974, Kowen et al 1979). Serological evidence, in the form of an elevated maternal antibody titre may indicate that an infection occurred at some time but does not show when it occurred, and has led to diametrically opposed conclusions (Lolis et al 1978, Johnson et al 1979). In order to diagnose an infectious aetiology of abortion both bacteriological, virological and histological evidence is required. Even when these parameters have been documented, a definitive causal relationship cannot always be established or ruled out.

Although anecdotal data indicate that organisms are capable of causing individual pregnancy losses, the data regarding the role of maternal and fetal infection in recurrent spontaneous abortion remains controversial. Several conditions are necessary for a microorganism to cause recurrent pregnancy loss. The organism needs to persist for long periods and produce minimal symptoms to escape diagnosis and treatment. In prospective reports, only a limited number of genital organisms have been cultured in a single study. In many reports, adequate control groups have not been used.

1.3.c.i. Bacteria and other micro-organisms.

Listeria.

Listeria monocytogenes is a microaerophilic gram positive rod of low pathogenicity present in animals and contaminated soil. In the non-compromised host it rarely causes serious illness but is an occupational hazard for abattoir workers, and may be acquired orally from contaminated foodstuffs especially unpasteurised dairy products. Most infections occur in patients with altered cell-mediated immunity secondary to pregnancy, malignancy and cytostatic drugs. That 58% of culture proven listeria infections occur in pregnant women (Seeliger et al 1968) has led to the suggestions that the organism has an affinity for the fetus and placenta and may be a cause of recurrent abortion. The fetus can acquire the organism during maternal bacteraemia (characterised by monocytosis) or rarely as a result of ascending genital infection, usually in the presence of intact membranes. The mother may be asymptomatic but more frequently records a mild flu-like illness in the second and third trimesters of pregnancy, with fever, myalgia, malaise, nausea and diarrhoea leading to late spontaneous abortion. Occasionally the disease may be fulminant with high maternal fever, abdominal pain and clinical chorioamnionitis. Treatment of symptomatic maternal disease with ampicillin is advocated (Watts & Eschenbach 1988) even though the evidence that listeria is of causal significance in abortion is conflicting (Editorial Lancet 1980).

Associations between listeriosis and recurrent pregnancy loss have usually been based on a positive culture in the index pregnancy with information on pregnancy loss derived from a retrospective history (Ochlschlager 1960, Desdepova et al 1978). Rappaport et al (1960) isolated the organism from 25 of 34 women with recurrent abortion but not from 87 comparable women with no history of repeated abortion. In contrast, other

studies have failed to report an association between bacteriological or serological evidence of listeria and sporadic or recurrent abortions (MacNaughton 1962, Rabau & David 1963). Furthermore, in one study *listeria* was isolated from the faeces of 35% of healthy pregnant women (Kampelmacher 1979). Chronic vaginal carriage of listeria has not been well documented and proof that listeriosis is a cause of recurrent abortion in humans awaits a carefully controlled study .

Brucella.

Brucella species were previously considered as a possible cause of spontaneous abortion in humans because of their association with infertility and pregnancy loss in animals, specifically *B.abortus* (cattle), *B.melitensis* (goats) and *B.suis* (hogs). Humans become infected with these small gram negative cocco-bacilli either through occupational exposure or through ingestion of unpasteurised dairy products. Patients with brucellosis typically have periodic nocturnal fevers, headaches, myalgias and weight loss. Sporadic cases of spontaneous abortion associated with human infection have been reported (Porreco 1974, Sarram et al 1974, Young 1983), but since *B.abortus* is being eradicated from cattle in Britain (Editorial BMJ 1981) it would be expected to be a rare cause of pregnancy loss. The difference in pathogenicity in human and animal abortions may be due to differences in placental metabolism. *Brucella* growth is stimulated by erythritol, an alcohol present in chorionic tissues of cows, sheep, pigs and goats but absent from human placental tissue. Clearly, animal data regarding infectious causes of recurrent pregnancy loss cannot be extrapolated to humans.

Campylobacter.

The campylobacters, previously known as *Vibrio fetus*, are associated with infertility, abortion and gastroenteritis in both animals and humans (Simor et al 1986). Typically maternal illness is mild with low-grade fever and mild diarrhoea, followed days to weeks later by spontaneous abortion or preterm labour. Campylobacter species can be recovered from maternal stool, the vagina and the fetus, but the relative contribution of Campylobacter infection to sporadic and recurrent pregnancy loss has not been quantified. A study of rectal cultures from 12,521 pregnant women yielded an 0.2% positive culture for *C.jejuni/coli* (Youngs & Roberts 1985) and symptomatic infection during pregnancy was rare. Isolated case reports of Campylobacter septicaemia leading to second trimester abortion are found (Gilbert et al 1981) especially in women with recent episodes of infectious diarrhoea. However the

contribution of these organisms to abortion must be small, and no evidence exists to link *Campylobacter* infection with repeated pregnancy loss in the normally immunocompetent pregnant woman.

Chlamydia.

The role of *Chlamydia trachomatis* as a causative agent in human spontaneous abortion has not been adequately documented, although bovine pregnancy loss with *C.psittaci* is well recognised. *C.trachomatis* is frequently isolated from the cervix in women examined for sexually transmitted diseases (Schacter et al 1975) and can be recovered from 3 -30% of pregnant women (Watts and Eschenbach 1988). In a small study by Martin et al (1982) an increase in late second trimester abortion was associated with the presence of Chlamydia detected before 19 weeks gestation but there are no studies that adequately address the role of *C.trachomatis* in first and early second trimester pregnancy losses. An association between Chlamydial infection and prematurity has been reported (Berman et al 1987, Gravett et al 1986) in particular when acute infection is diagnosed by a specific IgM antibody response during pregnancy (Harrison et al 1983).

However, no increase in the incidence of positive cultures among women with a history of repeated abortion has been reported, and the tendency for adverse pregnancy outcome to be more closely associated with the initial rather than the persistent chlamydial infection (Harrison et al 1983) argues against a significant role for this organism in recurrent abortion. A subgroup of patients may be at risk of recurrent losses due to recurrent sexual exposure and reinfection but since Chlamydia are found so commonly in the cervix it is doubtful whether cervical cultures, or culture of abortus material that has traversed the cervix from women with recurrent abortion will provide evidence of an aetiological role for this organism.

Mycoplasmas.

The two most common genital isolates are *Mycoplasma hominis* and *Ureaplasma urealyticum* (T-strain mycoplasma). Colonization is directly related to sexual activity and 40-70% of all women are vaginal or cervical carriers (Stray-Pedersen et al 1978). In the first trimester of pregnancy, *U. urealyticum* can be isolated in 40-90% and *M. Hominis* in 40-60% of women. The organisms are of low virulence but can produce postpartum fever (Taylor-Robinson & McCormack 1980).

An association between spontaneous abortion and Mycoplasma has been suggested by several authors who found that recovery of this organism

from fetal tissue spontaneously aborted was significantly higher than in tissue recovered from induced abortions in both the first and second trimesters of pregnancy (Kundsin & Driscoll 1970, Horne et al 1974, Sompolinsky et al 1975). However, the isolation of mycoplasmas from fetal tissues may simply reflect secondary infection of the necrotic products of conception (given the high prevalence of genital mycoplasma colonisation) rather than a primary fetal infection causing fetal death. Most prospective studies have not documented an increased rate of spontaneous abortion among women with a positive mycoplasmal culture before conception (Gump et al 1984) or at the first prenatal visit (Foy et al 1970, DiMusto et al 1973, Thompson et al 1982, Harrison et al 1983).

In vitro increases in chromosome breaks and tetraploidy have been noted in cell cultures infected with *U.urealyticum* recovered from patients with abortion and infertility compared to uninfected cultures (Kundsin et al 1971), but similar in vivo effects on embryonic tissue have not been demonstrated.

Despite their high frequency and low virulence, the role of genital Mycoplasma infections in recurrent abortion is unproven. Horne et al (1974) suggested that Ureaplasma may cause chronic endometritis leading to recurrent pregnancy loss. Improved pregnancy outcome in women with recurrent abortion treated with tetracycline regimens have been reported by several groups (Driscoll et al 1969, Horne et al 1974, Quinn et al 1983). Stray-Pedersen et al (1978) noted no difference in cervical colonisation rates, but isolated ureaplasma significantly more frequently from the endometrium in women who had a history of spontaneous abortion (28%) or infertility (50%) than among control patients (7%). Of 19 patients with positive cultures treated with doxycycline, 16 had a term delivery in the next pregnancy, but no untreated control group was included. Furthermore, controls for the large number of other potential organisms that would be inhibited by broad spectrum antibiotics therapy have not been included in these studies. Until well designed placebo controlled treatment studies in which a variety of genital organisms are cultured the use of antibiotics for treatment of mycoplasmas in recurrent pregnancy will remain empirical.

Treponema pallidum.

Untreated maternal syphilis is associated with increased pregnancy loss and was described in French literature as "la plus grande avortéuse". The risk of pregnancy loss and congenital fetal infection (which is acquired

transplacentally), is highest during the early stages of maternal disease and progressively decreases over time, amounting to almost 100% when infection occurs shortly before conception, following which spontaneous abortion is common. In the past, *T.pallidum* was not thought to cross the placental barrier before 16 weeks gestation, since neonates of mothers treated prior to this point in pregnancy showed no manifestations of congenital syphilis. However, Harter and Benirschke (1976) demonstrated the organisms in fetuses aborted at 8 to 9 weeks gestation. The absence of fetal syphilitic lesions before 16 weeks gestation is now believed to be related to the inability of the fetus to mount an appropriate inflammatory response before this time.

Syphilis is a proven infectious cause of recurrent pregnancy loss, and can be easily diagnosed and treated. Its contribution to recurrent abortion in developed countries where widespread serologic screening is performed is small.

Other bacteria.

Pregnancy loss associated with the gastrointestinal pathogen *Salmonella typhi* is thought to be primarily related to the severity of the maternal febrile illness (Wing & Troppoli 1930). Since specific serum antibodies are not protective against *S.typhi*, prolonged infection and multiple bacteraemic episodes are common, during which infection of placental and fetal tissue may occur. Infection early in gestation usually leads to spontaneous abortion, but in later pregnancy the infected fetus is usually asymptomatic (Sengupta et al 1980). Although chronic maternal carriers may have persistently positive faecal cultures, bacteraemia is rare after the third week. Thus although *S.typhi* may cause sporadic pregnancy loss, repeated pregnancy loss related to this organism must be rare.

Early spontaneous abortion has been associated with *Candida albicans* in patients with an intra-uterine contraceptive device (Ho & Aterman 1970, Hood et al 1983).

1.3.c.ii. Parasites.

Toxoplasma.

Toxoplasma gondii is acquired by inadequately cooked infested meat or by the inhalation of oocytes from cat faeces. Fetal infection occurs only when the mother acquires her primary infection during gestation (Desmonts & Couvreur 1974). There is no evidence that latent *Toxoplasma* infection in healthy mothers causes congenital infection or abortion (Lee 1988). The risk

and severity of fetal infection depends on the gestational age of the pregnancy. The incidence of intrauterine infection is highest when a non-immune mother is infected during the third trimester and may result in congenital disease or subclinical infection. Severity is greatest when infection occurs in the periconceptual period or in the first trimester and may result in spontaneous abortion, later intra-uterine death or liveborn children with severe neurologic lesions (Desmonts et al 1985).

The frequency of early abortion is probably not high (Kimball et al 1971, Johnson et al 1979, Stray-Pedersen 1980, Desmonts & Couvreur 1974), but the results of most of these studies have been based on a single method for the diagnosis of infection, and only a few have combined serological data and animal inoculation of gestational material. Using haemagglutination and immunofluorescent antibody tests together with quantitative immunoglobulin and mouse inoculation studies, Lolis et al (1978) evaluated 152 spontaneous abortions and concluded that toxoplasmosis should be considered as a cause of abortion only when the patient's IgG antibody titre exceeds 1 in 256.

The recent prospective study of 746 documented cases of maternal toxoplasma infection reported by Daffos et al (1988) used a combination of fetal blood sampling (IgM and IgG levels), mouse inoculation studies and detailed ultrasound scanning to achieve an antenatal diagnosis of fetal infection in 39 of 42 cases. Six pregnancies ended in spontaneous abortion or intra-uterine death. All the mothers were treated with spiramycin during pregnancy, and if fetal infection was demonstrated pyrimethamine and sulfadiazine were added to the regime. This study concluded that prenatal diagnosis and therapy of congenital toxoplasmosis reduces the severity of the fetal disease and suggests that screening of all pregnant women should be performed routinely.

Although toxoplasmosis is frequently cited as a possible cause of recurrent abortion (Huisjes 1984), there is no evidence that chronic maternal toxoplasma infection leads to abortion, except in the rare situation of an immuno-incompetent mother experiencing reactivation. Kimball et al (1971) found an association between toxoplasma and sporadic abortion but no increase in recurrent pregnancy loss among women with positive toxoplasmosis dye test or complement fixation tests. Similarly, Southern (1972) compared pregnant women with and without a history of repeated abortion and found no difference in their toxoplasmosis dye testing. On the other hand, detection of the parasite by immunofluorescence has been reported more commonly in the endometrium and menstrual blood of

women with repeated abortions compared to controls (Stray-Pedersen & Lorentzen-Styr 1977). However, these results were not confirmed by animal inoculation studies and no correlation between the maternal antibody titres, endometrial biopsies or abortion history were made.

Although infection with *Toxoplasma gondii* is another cause of sporadic abortion, there are few data to support its role in recurrent pregnancy loss. Indeed, the realisation that toxoplasma only infects the fetus during the course of a primary maternal infection excludes it as a cause of recurrent abortion (Feldman 1974) and the routine screening of patients with recurrent abortion for this organism is seldom warranted. The purpose of preconception and prenatal screening of susceptible women is to reduce the risk of acute maternal infection, identify the affected mother soon enough to choose appropriate management options and thereby reduce the risk of congenital infection.

Malaria.

Malaria remains a major health problem in developing countries and has been widely held responsible for spontaneous abortion in endemic areas (Covell 1950). Pregnancy appears to increase a woman's susceptibility to the disease - both the prevalence and the density of the parasitaemia are increased in pregnant women compared to normal controls (Cannon 1958).

Spontaneous abortion may result during a severe attack of malaria in the first trimester, but it is unclear whether the abortion is related to direct infection of fetal tissue or to the fever and poor maternal health. The incidence of early and late abortion, prematurity and low birthweight are significantly higher in cases of placental infection (MacGregor & Avery 1974) and are dependant on the degree of tolerance possessed by the mother. Maternal antibody production may offer some protection against fetal infection, but women can have several pregnancies with recurrent placental and congenital malarial infections. Non-immune and highly immune women are at greatest risk, whereas the moderately immune mother appears to passively protect her fetus.

1.3.c.iii. Viruses.

Viral infections during pregnancy are extremely common. They may be more severe and prevalent amongst pregnant women, but it is not clear whether it is the increased susceptibility to the organisms or histiotropism for the placenta and fetus that is responsible for intra-uterine infection. In the majority of cases the fetus is left unharmed and the pregnancy continues

normally (Waterson 1979). Well known exceptions to this rule are Rubella and Cytomegalovirus infections. However, the fact that these 2 viruses are an important cause of congenital abnormality, does not necessarily imply that they often lead to abortion.

The incidence of many viral diseases has been reduced by vaccination and therefore epidemiological data is difficult to obtain. Direct evidence of a causal relationship by virus isolation from products of conception is not as easily obtained as in bacterial infections (Alberman 1973). Furthermore the temporal relationship between viral infection and abortion is not always clear, the viraemia and resultant fetal infection may be occult or precede any clinical manifestation of the disease. "Fever" during pregnancy has been associated with an increase in euploid but not aneuploid abortions when compared to control women "without fever" (Kline et al 1985). In one study by Lauritsen (1976) maternal influenza infection occurring after the last missed period was implicated as a cause of early spontaneous abortion based on the morphological characteristics of abortus material. However in this study no attempt was made to cultivate virus from the conceptus.

Cytomegalovirus.

CMV is the most common viral infection transmitted to the fetus. The risk of primary infection during pregnancy depends upon the population studied. Serological studies of pregnant women indicate that 50 to 80% of women have had primary CMV infections prior to pregnancy, the incidence being higher in lower socioeconomic groups (Stagno et al 1982). Of the remaining susceptible women, approximately 2% will develop a primary CMV infection during pregnancy. Primary infections are usually asymptomatic, although some women may develop a mononucleosis - like syndrome. Primary CMV infection during pregnancy may lead to spontaneous abortion (Altshuler 1974, Griffiths et al 1980) but the magnitude of the problem is not clear (Hanshaw & Dudgeon 1978).

In ongoing pregnancies complicated by primary CMV infection, approximately 40% of the infants will be culture positive at birth, of whom 10% will have clinical manifestations such as hepatosplenomegaly, jaundice thrombocytopenia, chorioretinitis, microcephaly and mental retardation (Stagno 1986). However, culture positive infants with no overt stigmata of disease are still at risk of sequelae. In total, 5-15% of infants with congenital CMV infection will develop significant hearing loss by the age of 2 years and

others are at risk for later developmental delays and learning disabilities (Reynolds 1974).

After the primary infection, CMV enters a latent stage and may be recovered from the urine and genital secretions of 5-15% of pregnant women. CMV is one of the few viruses that can produce a secondary viraemia, which occurs during the latent phase of infection and is usually asymptomatic. Although uncommon, recurrent in-utero infections have been reported (Stagno et al 1982) and CMV reactivation in early pregnancy may occur (Kriel et al 1970), although it is difficult to differentiate between infected tissue and contamination from asymptomatic cervical infection.

Rubella.

Primary infection during the first trimester of pregnancy may lead to spontaneous abortion or congenital infection with multiple fetal defects. The risk of fetal damage is highest with maternal infections occurring early in pregnancy and decreases progressively thereafter. Although several authors have reported a higher incidence of spontaneous abortion after rubella infection in early pregnancy (Manson et al 1960, Siegel et al 1966), precise figures are difficult to obtain since only 10-20% of adult rubella infections are diagnosed and reported (Horstmann 1971). Siegel et al (1966) recorded a 20% abortion rate in pregnancies complicated by rubella during the first 12 weeks compared with 13% in a control group. In countries where vaccination is practised, rubella is not a common complication of pregnancy, and since many of the diagnosed cases will undergo termination of pregnancy, the number of spontaneous abortions attributable to rubella infection are small. Maternal antibody production from primary infection or immunisation protects against subsequent fetal infection. Hence, recurrent pregnancy loss from rubella would not be expected and has not been reported (Watts & Eschenbach 1988).

Herpes.

There have been several reports of an increased rate of spontaneous abortion following primary *herpesvirus hominis* infection. Nahmias et al (1971) documented a 34% abortion rate in pregnant women developing herpetic infection before 20 weeks gestation compared to 11% in control subjects. Similar abortion rates were found by Naib et al (1970) who further noted that the increased incidence of spontaneous abortion was evident even when there was an interval of several months between the diagnosis of herpetic infection and pregnancy. Diagnosis in both these studies was based on cytological evidence and not confirmed in tissue culture. However, Grönroos

et al (1983) investigated 189 patients with threatened abortion and documented an association with herpesvirus using virus isolation and serum and cervical IgA antibody levels.

Although maternal antibody does not protect against local reactivation of maternal genital herpes, viraemia and consequent in utero infection of the fetus do not occur with latent or recurrent genital herpes infection. Thus, recurrent abortion would not be expected to result from maternal HSV infection, a view supported by the data demonstrating that the increase in spontaneous abortion only occurs in primary infections contracted in early pregnancy.

Parvovirus.

The parvovirus B 19 has now been implicated as a cause of intrauterine infection. Human parvovirus B19 infection runs a similar clinical course to rubella which has led investigators to look for evidence of congenital infection. In a study of 600 clinically suspected cases in the USA (Ager et al 1966) no increase in the number of congenital malformations was reported. In an outbreak in North Devon (Cramp & Armstrong 1977) women in the first trimester of pregnancy did not have an increased incidence of spontaneous abortion or congenital abnormality. To date, there is no accurate estimate available of the risk from B19 to the fetus. Brown (1989) reviewed 7 cases reported in the UK where maternal B19 infection between 7 and 16 weeks gestation led to hydrops fetalis and there is only one reported case of congenital malformation associated with B19 infection (Weiland et al 1987). Several small studies of women with serological evidence of B19 infection during pregnancy have reported an incidence of abortion in the second trimester of 30-40% (Anand et al 1987, Mortimer et al 1985, Schwartz et al 1988). However, there are at least 130 cases reported of favourable pregnancy outcome after confirmed parvovirus B19 infection in pregnancy (Kinney et al 1988).

It is hoped that the results of the Public Health Laboratory Service Working Party on B19 infection in pregnancy will clarify the risks to the fetus and improve management of those women who may have been infected. At the present time, when assessing a suspected case it is useful to remember that 23-45% of pregnant women are susceptible to B19 infection (Mortimer et al 1985). Therapeutic termination of pregnancy is not indicated since congenital defects have not been associated with B19 infection. At least 80% of pregnancies in which B19 infections occur result in normal live births. In

cases of proven B19 infection the monitoring of maternal serum for raised alpha-fetoprotein levels and serial ultrasonography have been recommended for assessing the risk of developing hydrops and fetal aplastic crises (Carrington et al 1987). In a very limited number of cases intrauterine exchange transfusion has been performed (Schwartz et al 1988). In conclusion, maternal B19 infection is not invariably transmitted to the fetus (Mortimer et al 1985, Woernle et al 1987) and the factors determining such transmission have not been elucidated. Pregnancies complicated by maternal B19 infection can result in miscarriage, stillbirth or a healthy live child. The fetal damage caused by B19 infection may vary with gestational age at the time of fetal infection (Elsacker-Niele et al 1989).

Other viruses.

In-utero transmission of Human Immunodeficiency Virus (HIV) is known to occur but at the present time insufficient data exist to link this virus with an increased risk of sporadic or recurrent abortion. In-utero transmission during the first trimester of pregnancy awaits confirmation from prospective studies.

Hepatitis A is quoted as being of causal significance in the aetiology of spontaneous abortion (Hanshaw & Dudgeon 1978), but there are no exact data on the association of either hepatitis A or B and abortion. Hepatitis was not associated with first trimester abortion in the prospective study by Siegel et al (1966).

There is no evidence to suggest that *varicella-zoster* virus leads to spontaneous abortion (Siegel et al 1966, Brazin et al 1979), although congenital abnormalities in the fetus are most severe when the disease is contracted in early pregnancy (Alkalay et al 1987). Infection with mumps virus occurs less frequently in pregnancy (Philip et al 1959), but the risk of spontaneous abortion is increased in those pregnant women who acquire the disease (Siegel et al 1966, Garcia 1980). Measles (*rubeola*) virus has been associated variably with an increased risk of abortion (Jespersen et al 1977, Siegel et al 1966)

Variola and *vaccinia* viruses have been associated with spontaneous abortion (Levine et al 1974) but vaccination has resulted in virtual extinction of smallpox and a recent report from Finland demonstrated that oral polio vaccination during pregnancy had no harmful effect on fetal development (Harjuhleto et al 1989).

Influenza infection in early pregnancy probably leads to an increased incidence of spontaneous abortion (Waterson 1979), but the possibility that the

abortion occurs secondary to maternal fever is supported by the lack of viral cultures in some studies (Lauritsen 1976).

Viral infections remain a possible cause of recurrent pregnancy loss. Certain viruses in which maternal antibody production during primary infection is protective against recurrent infection, such as rubella, would not be expected to cause repeated losses. However, the possibility of recurrent loss remains for infections that reactivate or persist despite maternal antibody production such as CMV and HIV.

In summary, some microorganisms cause spontaneous abortion and infection is a remediable cause of abortion if the patients can be identified and treated. Although some viral infections that reactivate or persist despite maternal antibody production (such as CMV) may cause recurrent abortion, little data exist at the present time to support the role of infection in the aetiology of recurrent early pregnancy loss and the results of studies claiming benefit from empirical drug regimens require cautious interpretation.

1.3.d. Maternal systemic disease.

Maternal systemic disease as a cause of spontaneous abortion is not common, even metabolic disturbances as severe as may occur in hyperemesis gravidarum do not appear to lead to abortion. However for some maternal diseases an association with recurrent abortion has been presumed because the causal factors are likely to be present in all of the woman's pregnancies.

1.3.d.i. Thyroid dysfunction.

Disorders of thyroid function have been considered a risk factor for spontaneous abortion (Lauritsen 1977, Winikoff & Malinek 1975). Many clinicians still consider evaluation of thyroid function obligatory in patients with recurrent abortions, but there is no firm evidence to support this view. Hypothyroidism has been reported to cause infertility and an increased rate of fetal loss should pregnancy occur (Greenman et al 1962, Jones & Man 1969, Naumoff & Shook 1963).

Winikoff & Malinek (1975) assessed thyroid function in normal pregnant women and pregnant women with a history of recurrent abortion all of whom were clinically euthyroid. They used a thyroid test "profile" to investigate their patients and reported that normal women reached a typical thyroid profile at 7-8 weeks gestation, while patients with a history of abortion

who carried a pregnancy to term did not reach this profile until 14-15 weeks. In recurrent aborters who miscarried again the test profile was always low, and four of these patients were treated with thyroxine, following which the lag response returned to normal and the pregnancies were carried to term. However, the small numbers in this study do not justify the suggestion that euthyroid recurrent aborters should be treated with thyroxine. There is no evidence to implicate hyperthyroidism as an important cause of abortion (Prout 1975, Reynolds 1978).

1.3.d.ii. Diabetes.

There have been few controlled studies of the risk of spontaneous abortion associated with diabetes, although there is evidence that the offspring of poorly controlled diabetic women are at increased risk for perinatal mortality and congenital malformations (Dekaban & Baird 1955, Drury et al 1977, Miodovnik et al 1984). Abortion rates of 25-30% have been reported in uncontrolled series of diabetic patients (Wright et al 1983, Miodovnik 1984). In one controlled study an increased incidence of abortion before and after diagnosis was noted (Dekaban & Baird 1955). However, similar rates of abortion in women with gestational or frank diabetes compared to controls and no increase in the incidence of recurrent abortion were reported by Crane and Wahl (1981). The disparity between the two studies could be due to the improved treatment of diabetes in the later series.

Support for this viewpoint comes from the observation of Wright et al (1983) who found higher levels of glycosylated haemoglobins (a measure of long term glucose control) in diabetics whose pregnancies ended in early spontaneous abortions, compared with diabetics who aborted late in gestation or delivered after 28 weeks gestation. In a recent prospective study, the incidence of clinical spontaneous abortion in insulin dependant diabetic pregnancies was reported as 30%. The increased risk of spontaneous abortion was correlated with poor metabolic control around conception and early weeks of pregnancy, together with decreased maternal magnesium levels (Miodovnik et al 1988).

Nearly all recently reported series of habitual abortion have failed to demonstrate diabetes mellitus as a significant aetiological factor (Tho et al 1979, Harger et al 1983, Stray-Pedersens 1984), suggesting that routine glucose tolerance testing in the evaluation of patients with recurrent abortion is not informative.

1.3.d.iii. Systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is the most common autoimmune disorder found in women of childbearing years (Scott 1979, Lubbe & Liggins 1988). It is now generally accepted that fertility is preserved in women with SLE (Fraga et al 1974) and that reproductive failure in these women is due to an increased rate of fetal loss. Sporadic and recurrent abortion, intra-uterine growth retardation and death and preterm labour have all been reported.

Several mechanisms have been proposed to account for the fetal losses observed. The serum of women with repeated fetal losses may contain a number of autoantibodies: the anti-phospholipid antibodies (lupus anticoagulant and anti-cardiolipin), anti-nuclear antibody and anti-Ro(ss-A) antibody (Petri et al 1987, Tincani et al 1987, Hull et al 1983), which may be deposited as immune complexes at the trophoblast basement membrane (Grennan 1978), cross react with trophoblast antigens (Bresnihan et al 1977) or in the case of anti-Ro antibodies, be passed transplacentally to the fetus (Scott et al 1983).

However there is considerable evidence to suggest that it is the anti-phospholipid antibodies, cardiolipin and lupus anticoagulant which have the greatest influence upon fetal loss (Lockwood et al 1986, Lubbe & Liggins 1985, 1988, Derue et al 1985, Cowchock et al 1985). The most popular explanation is that antiphospholipid antibodies cause thrombosis of placental vessels resulting in placental infarction and fetal death (Abramowsky et al 1980, Derue et al 1985, De Wolf et al 1982, Lockshin et al 1985). Thrombotic episodes involving both the arterial and venous circulations occur in 30% - 50% of individuals with lupus anticoagulant and are the commonest non obstetric clinical manifestation of these antibodies, which are thought to inhibit prostacyclin production with a resultant increase in levels of thromboxane, which is a potent vasoconstrictor and platelet aggregator (Carreras et al 1981a).

Although the lupus anticoagulant (LA) was first identified in the plasma of SLE patients (Conley & Hartmann 1952) many individuals with the lupus anticoagulant do not have overt SLE. It is now recognised that within this group fall a number of women whose first presentation is with recurrent spontaneous abortion (Cowchock et al 1984). While some of these recurrent aborters develop clinical signs of SLE at a later date, many have no manifestations of autoimmune disease when not pregnant.

Incidence of fetal loss in SLE.

Most authors agree that the majority of women with the lupus anticoagulant suffer recurrent fetal losses. A survey of the literature suggests that 89% of women with circulating anticoagulant have had one or more fetal losses (Branch et al 1985). Perinatal survival rates are low in most reports ranging from 3% to 14% (Lubbe et al 1983, Elias & Eldor 1984, Prentice et al 1984, Branch et al 1985) although not all reports have painted such a gloomy picture (Lockshin et al 1987, Ros et al 1983). Similarly, there is a high correlation between women with positive anticardiolipin tests and fetal loss (Derue et al 1985, Lockshin et al 1985, Lockwood et al 1986). In a study of 121 women with varying IgG anticardiolipin levels Harris et al (1986) found that the incidence of fetal loss increased as the level of anticardiolipin increased.

The prevalence of women with anti-phospholipid antibodies is uncertain. In an unselected population of women with recurrent abortion the incidence is probably about 10% (Tincani et al 1987, Petri et al 1987, Lockwood et al 1986), but if women with recurrent fetal loss associated with placental infarction and maternal thrombotic episodes are appropriately investigated a significantly higher yield of positive tests are noted, reaching 100% in some studies (Soulier & Boffa 1980, Firkin et al 1980, Carreras et al 1981b, Lubbe et al 1984). Fetal loss can occur at any stage in pregnancy but most authors report the majority in the first trimester (Derue et al 1985, Branch et al 1985). Furthermore there appears to be a trend for progressively earlier losses to occur in the same patient. In the series of Lubbe et al (1984) women who had their first fetal loss during the second or third trimester invariably had subsequent abortions in the first trimester, suggesting that treatment should be started early in future pregnancies.

Screening for SLE.

The lupus anticoagulant is an acquired IgM or IgG antiphospholipid antibody and is recognised by its ability to prolong phospholipid dependant coagulation tests. By competing with the binding of factors Xa and Va to platelet wall phospholipid matrix in the presence of calcium ions, it interferes with the formation of the prothrombin activator complex which catalyses prothrombin to yield thrombin. The tests commonly used for detecting the anticoagulant are the partial thromboplastin time (APPT) the Russell Viper Venom time (RVVT) and the kaolin clotting time (KCT). The KCT is believed to be the most reliable and sensitive test (Exner et al 1978). Since various clotting factor deficiencies also prolong these tests, the presence of the lupus

anticoagulant is confirmed by the addition of normal plasma. If the circulating inhibitor is present the clotting time remains prolonged.

Cross reactivity has been demonstrated between lupus anticoagulant, anti-cardiolipin and anti-nuclear antibody. The development of a radioimmunoassay for anticardiolipin antibody (Harris et al 1983, 1985) has demonstrated that the vast majority of patients with lupus anticoagulant also have positive anti-cardiolipin tests. However the correlation is not complete, several investigators have reported patients whose plasma is positive for the anticoagulant and negative for cardiolipin antibodies (Lockshin et al 1987). Similarly, high cardiolipin titres are not invariably accompanied by prolongation of clotting tests, which seems paradoxical when it is recalled that only about 30% of lupus anticoagulant positive patients are subject to thrombosis, whereas 70% or more of the anti-cardiolipin positive patients are predisposed to thrombosis (Harris et al 1986). Dissociation between these two phospholipid antibodies can also be demonstrated after steroid treatment. Anticoagulant activity can disappear altogether while cardiolipin titres remain relatively unaltered (Derksen et al 1986).

Table 1.2.

Prevalence of autoantibodies in lupus anticoagulant positive obstetric patients (Lubbe & Liggins 1988)

Autoantibodies	No. of patients	% Positive tests
Lupus anticoagulant	25	100
Anticardiolipin	25	100
Antinuclear	22	88
Anti-DNA	5	20
Anti-ssDNA	9	36
Anti-smooth muscle	13	52
Anti-Ro	2	8

The recent availability of effective treatment for patients with recurrent abortion has resulted in the need for screening at risk patients early in pregnancy. Although clotting studies (KCT) are the most reliable method of diagnosis, they are time consuming and expensive. The anti-nuclear antibody test has been proposed but only 60-80% of patients with lupus anticoagulant

have positive tests, and the VDRL test which is easy to perform is positive in only 20-30% of women (Lubbe & Liggins 1988).

The most promising screening test appears to be the assay for anticardiolipin antibody, using sensitive automated procedures developed by Harris and colleagues (1985b, 1987), which can be performed on stored serum. Positive anticardiolipin antibody tests should be followed by definitive anticoagulant screening. Table 1.3 lists the indications for investigation of obstetric patients for the presence of lupus anticoagulant.

Table 1.3.

Indications for investigation of obstetric patients for presence of lupus anticoagulant.

Fetal losses - recurrent first trimester,
late losses associated with placental infarction
Intrauterine growth retardation
Clinical suspicion or signs of autoimmune disease
Deep venous or arterial thrombosis
Thrombocytopenia in pregnancy
Pre- eclampsia- especially if atypical or recurrent
Pulmonary hypertension
Chorea gravidarum
Positive antinuclear antibody test
Positive anticardiolipin antibody test
Biologically false positive VDRL test

Treatment of SLE.

The first successfully treated pregnancies in women with lupus anticoagulant and multiple fetal losses, using steroids and low dose aspirin, were reported by Lubbe et al in 1983 and 1984. Since that time several other centres have employed similar treatment regimes and success rates of 65% - 85% have been reported although the patient numbers are small (Farquharson et al 1984, Vermynen et al 1986, Branch et al 1985). In order to minimise the number of women subjected to the hazard of high dose steroid therapy, Lubbe and Liggins (1988) have confirmed the efficacy of low-dose aspirin treatment alone for women with a mild prolongation of the KCT (one and a half times control). If steroids are required to suppress higher KCT values, prednisone

and aspirin are started as soon as pregnancy is diagnosed and the prednisone dosage increased if the KCT remains prolonged. The aim of treatment is to achieve a normal KCT value during the gestational period 18 to 24 weeks, the time recognised as most important for placentation and fetalisation of maternal spiral arterioles to proceed normally.

Ultrasound assessment demonstrating favorable fetal growth and placental texture patterns that do not show increasing echodensity have been found useful in identifying those pregnancies in which steroid dosage can be reduced after 28 weeks, irrespective of the KCT result at this stage. Other forms of treatment such as azathioprine, high dose immunoglobulins and subcutaneous heparin in women with a past history of venous thrombosis have also been reported (McVerry et al 1985, Gregorini et al 1986, Chan et al 1986).

1.3.d.iv. Other maternal medical disorders.

Maternal congenital cardiac disease has been associated with very high rates of pregnancy loss, exceeding 50% in one study (McAnulty et al 1982). A deficiency in the supply of well-oxygenated blood to the developing fetus may be the factor responsible. Renal disease, especially when associated with hypertension carries a high risk of abortion, but moderate renal insufficiency, regardless of its cause, appears to have little effect on the fetus if hypertension is not present (Zacur & Mitch 1977, Katz et al 1980).

There is very little data regarding the effect of drug treatment during pregnancy on the incidence of spontaneous abortion. In a survey of the literature Hall et al (1980) found evidence that coumarin derivatives used during the first trimester of pregnancy were associated with an increased incidence of abortion. The increased risk of fetal loss among women suffering from migrainous headaches has been causally linked to the use of ergotamine preparations but this data is uncontrolled (Wainscott et al 1978). Several studies have investigated the relationship between epilepsy, anti-convulsants and congenital malformations (reviewed by Strobino et al 1978), few studies have been concerned with spontaneous abortion. Meyer (1973) found an increased rate of abortion in untreated epileptics compared to women taking anticonvulsants, but other studies have found no difference in the abortion frequency among epileptics compared to controls (Speidel & Meadow 1972, Knight & Rhind 1975)

Patients with Wilson's disease (disturbed hepatic copper metabolism) invariably abort their pregnancies although the causal mechanism is not understood. When treated with penicillamine, which is not teratogenic, the pregnancy usually proceeds normally. Although Wilson's disease is rare, a serum caeruloplasmin measurement in unexplained cases of recurrent abortion may be indicated (Walshe 1977, Klee 1979).

1.3.e. Endocrine.

Ovulation, implantation and the early stages of pregnancy are dependant upon an intricate maternal endocrine regulatory system. As discussed earlier (Section 1.2.a; p 13-16), the vast majority of embryos fail to survive the first 4 weeks following fertilisation, and this high incidence of early pregnancy loss is believed to be largely due to genetic abnormalities of the conceptus. However, the extent to which inadequacies in the maternal endocrine system may contribute to pregnancy failure when the embryo is capable of normal development are poorly understood.

Progesterone deficiency as a likely cause of abortion was generally assumed after Allen and Corner (1929) published their investigations on the physiological properties of the corpus luteum in 1929. The product of the corpus luteum, progesterone, is the principle factor responsible for the conversion of a proliferative to a secretory endometrium, a transition that in some way renders the endometrium receptive to the arrival of the conceptus. Under the continued influence of progesterone, the secretory endometrium produces a number of specific proteins that are probably crucial to the implantation process (Martin 1980, Joshi 1983). Progesterone may also inhibit uterine prostaglandin production (Kennedy 1980) and has been implicated as a factor preventing immunological rejection of the embryo (Lee 1987).

The duration of the luteal phase in the nonfertile menstrual cycle varies between 10-15 days. The mechanism responsible for spontaneous regression of the corpus luteum (luteolysis) remains unknown despite extensive investigation, but in fertile cycles, corpus luteum function is "rescued" or prolonged by chorionic gonadotrophin (hCG) produced by the trophoblast.

When progesterone is abruptly withdrawn by oophorectomy or lutectomy during early pregnancy (Csapo et al 1973), or when a patient is treated with the progesterone antagonist RU-486 (Baulieu 1985), pregnancy is abruptly terminated. Luteal support remains essential until approximately the

seventh week of gestation, presumably the time when the trophoblast has acquired sufficient steroidogenic capacity to support the pregnancy itself. In lutectomised patients, treatment with parenteral progesterone prior to the time of luteoplacental shift in progesterone synthesis prevents abortion (Csapo 1973). Removal of the corpus luteum after this date does not induce abortion necessarily (Csapo 1972).

1.3.e.i. The Corpus luteum deficiency syndrome.

The concept of "corpus luteum deficiency" (CLD) was first introduced by Jones (1949) who noted that insufficient progesterone production resulted in a spectrum of clinical presentations including infertility, failed implantation and early pregnancy loss (Jones 1975, Wentz 1979, Baird et al 1975). CLD may be associated with specific endocrine abnormalities such as hyperprolactinaemia and hypothyroidism and certain aspects of follicular development clearly predetermine the functional adequacy of the corpus luteum. There must be (1) sufficient numbers of granulosa cells in the follicle before ovulation with (2) the capacity to secrete sufficient progesterone after ovulation and (3) the granulosa and theca cells must be responsive to gonadotrophin stimulation with follicle stimulating hormone (FSH) and luteinising hormone (LH) (McNatty et al 1975). However, although CLD is often a sequel to defective folliculogenesis (DiZerega & Hodgen 1981) it may also follow normal ovulation and develop as a consequence of specific luteal phase abnormalities at hypothalamic-pituitary, ovarian or endometrial sites (reviewed by Fritz 1988).

Hence the mechanisms of CLD in the infertile patient and in the habitual aborter may be different. Infertility is generally believed to result from abnormal or delayed development of the secretory endometrium with disruption of normal implantation. In the recurrent aborter luteal function is apparently sufficient to promote endometrial maturation but is quantitatively inadequate to support the pregnancy during the transitional period of the luteoplacental shift. However, these two manifestations of reproductive failure may represent two ends of a spectrum of disease, both of which start with an abnormality in follicular development (see PCOS; p 72). Patients with apparent infertility may be aborting subclinically since occult pregnancies have been identified in such women screened for plasma β -hCG during the later stages of the luteal phase (Cline 1979, Chartier et al 1979). Recurrent aborters

have been shown to have a higher incidence of relative infertility when compared to the general population (Strobino et al 1986).

Incidence of corpus luteum deficiency.

In general, a deficient luteal phase can be found in approximately 10% of women undergoing infertility investigations, the diagnosis being made significantly more often in certain subgroups of the infertile population (advanced age, hyperprolactinaemia, polycystic ovarian disease, ovulatory induction therapy). The frequency among women with repeated pregnancy failure is estimated to be much higher, ranging from 23% to 60% (see Table 1.4). Since there is no reliable method available currently to recognise inadequate luteal function in the pregnant patient, evaluation of patients with recurrent abortion have focused on the corpus luteum of the non-fertile menstrual cycle, despite evidence that the corpus luteum of pregnancy and that of the non-fertile cycle are different entities (Stouffer 1988).

Diagnosis of corpus luteum deficiency.

Basal body temperature - An elevation of basal body temperature (BBT) of less than 11 days before the onset of menses or a slow or limited thermal shift has been used to diagnose CLD (Jones 1949, Andrews 1979). However, a short luteal phase may be observed in 10-18% of cycles in women with proven fertility (Marshall 1963, Smith et al 1984), and although the mean length of the luteal phase is significantly shorter in deficient compared to normal cycles, most luteal phases are still of normal duration (Downs & Gibson 1983, Lenton et al 1984) and may even be prolonged (Leyendecker 1979).

Progesterone estimations - Low progesterone levels (Strott et al 1970) and an inadequate rise of BBT (Leyendecker 1979) have been demonstrated in women with a short luteal phase compared to controls, which has led to the suggestion that the amount of progesterone secreted by the corpus luteum is an indication of its functional capacity. A wide range of progesterone levels representative of normal luteal function, ranging from 3 ng/ml to 10 ng/ml (9-30 nmol/l) in non-fertile menstrual cycles (Johanssen 1969, Hensleigh & Feinstat 1979, Landgren 1980, Israel 1972, Radwanska et al 1981) and cycles of conception (Tulchinski & Hobel 1973, Sobowale et al 1978, Hull et al 1982) have been reported. This variation may be due to differences in assay technique, the choice of study patients and pulsatile fluctuations of hormone within the cycle (Radwanska & Swyer 1974). Since the interpretation of a single mid-luteal phase progesterone measurement is uncertain, Abraham

(1974) suggested that CLD may be diagnosed if the sum of three samples obtained 4 to 11 days premenstrually is less than 15 ng/ml (45nmol/l).

CLD with low progesterone levels has been documented in women with recurrent episodes of abortion (MacNaughton 1976, Yip & Sung 1977). Horta et al (1977) found significantly lower luteal progesterone levels in 15 women with a history of three or more abortions, compared to women with normal obstetric histories. Of the women from the study group who became pregnant subsequently, all aborted again. Among 66 women hospitalised in early pregnancy for threatened abortion, patients who subsequently aborted had progesterone levels significantly lower than values found in normal pregnancy or in patients with threatened abortion who did not abort (Radwanska 1978).

However, there is no reliable method to diagnose CLD once pregnancy is established since plasma progesterone concentrations represent both corpus luteum and trophoblast production. Simultaneous measurement of hCG will not differentiate the deficient source of progesterone production since low levels of hCG may reflect an abnormal conceptus which stimulates less progesterone production from an otherwise normal corpus luteum (which may explain the progesterone deficiency associated with ectopic gestations (Hubinont et al 1987)). Conversely, a decline in the rate of hCG production may be secondary to a decompensating corpus luteum and the loss of gestational support to an otherwise normal pregnancy. Hence, the reliability of progesterone determinations in the evaluation of luteal function is even more limited in early gestation than during the nonfertile menstrual cycle.

Endometrial biopsy - Although luteal phase duration and circulating progesterone concentrations are indicators of luteal function, these methods do not reflect the effect of progesterone on the endometrium, which may be responding inappropriately to a normal progesterone stimulus (Jones 1976). Theoretically endometrial biopsy should identify abnormalities resulting from both deficient corpus luteum steroid production and endometrial response.

Evaluation of luteal function by endometrial biopsy has been used widely to diagnose CLD among infertility patients (Shepard & Senturia 1977, Rosenberg et al 1980) and patients with repeated abortion (see Table 1.4; p 70). Using the histological criteria described by Noyes et al (1950) to date the endometrium, a biopsy from the uterine fundus within 2 to 3 days of the expected menses is assigned a histologic date, based on the sequence of

endometrial maturation that characterises the normal luteal phase. The diagnosis of CLD is made by dating the endometrium 2 or more days out of phase with the subsequent period (Jones 1975). Abnormal results in two separate cycles are required in order to avoid false positive results from isolated short cycles in normally fertile women (Jones 1976, Wentz 1979, 1980).

Although Jones (1976) and Wentz (1980) believe that the results of the endometrial biopsy are both diagnostic of a CLD and prognostic for future pregnancy outcome, other workers have failed to find this method so reliable (Cooke et al 1972, Rosenfeld & Garcia 1976). Shepard and Senturia (1977) found a better correlation between progesterone levels and the onset of the next

Table 1.4.

Incidence of Corpus Luteum deficiency among patients with recurrent abortion.

Authors	No.of patients	% CLD diagnosed by endometrial biopsy
Jones & Delfs 1951	73	37
Grant et al 1959	170	60
Botella-Lhuisa 1962	50	38
Jones et al 1968	120	28
Yip & Sung 1977	10	30
Tho et al 1979	100	23
Wentz 1980	8	38
Balasch et al 1986	60	28

menstrual period than between the biopsy and the onset of menses. Interestingly, although endometrial biopsies performed in conception cycles do not appear to increase the incidence of abortion, the information obtained from the biopsy provides no predictive information about the outcome of the pregnancy (Wentz et al 1986).

Other methods - Since the above three methods for assessing the adequacy of luteal function have significant shortcomings, the search continues for a more reliable parameter that will detect deficiencies of both steroid production and endometrial response yet be relatively noninvasive and independent of subjective interpretations. Progestin dependant

endometrial protein (PEP) may become a useful biochemical marker of progesterone induced secretory endometrial development (Joshi 1983). PEP appears to be a secretory product of the endometrial glands during the late luteal phase and can be measured in the peripheral circulation. Levels rise steeply in the late luteal phase after circulating progesterone concentrations have already begun to decline. During early pregnancy PEP levels continue to rise and mirror the pattern of circulating hCG concentrations. A similar pattern has been described in concentrations of placental protein 14 (Julkunen 1986a) which is now known to be immunologically indistinguishable from PEP (Julkunen 1986b).

A number of investigators have measured the endometrial steroid receptor profile of the luteal phase defect, in an attempt to rationalise treatment programmes, following reports that the infertility resulting from a deficiency of progesterone receptors is resistant to progesterone therapy (Keller et al 1979, Maynard et al 1983). Theoretically, an endometrium possessing a normal progesterone receptor population should respond to progesterone supplementation, whereas the receptor-poor endometrium does not (Levy et al 1980, McRae et al 1984, Jacobs et al 1987)

Treatment of CLD and progesterone deficiency.

There have been numerous anecdotal reports of the use of progesterone supplementation for the treatment of patients with threatened and recurrent spontaneous abortion. Vaginal suppositories (Aksel & Jones 1974, Jones et al 1974) or intramuscular injections continuing until the 12th week of pregnancy (Wentz 1979, Andrews 1979) have been shown to increase plasma progesterone levels. Although there is some evidence that correction of a CLD in the habitually aborting patient can improve subsequent pregnancy outcome (Jones & Delfs 1951, Jones 1975, Balasch et al 1986, Wentz et al 1984) no proof exists to support the efficacy of empiric hormonal therapy in an unselected group of women with repeated abortion. Shearman & Garrett (1963) demonstrated that recurrent aborters have a spontaneous salvage rate of 80% and Goldzeiher (1964) calculated that over 1000 recurrent aborters would be required to prove the value of a therapeutic agent which could raise the salvage rate from 80-90%. Several other large controlled studies have failed to show the benefit of hormonal supplementation in the treatment of recurrent abortion (Klopper & MacNaughton, McDonald et al 1972, Reijnders et al 1988).

The safety of progesterone therapy during early pregnancy has been examined extensively. Although synthetic progestins may have teratogenic

effects (Chez 1978, Andrews 1979) and actually suppress the activity of the corpus luteum (Johanssen 1971), there is no data to suggest that naturally occurring progesterone preparations increases the risk of congenital abnormalities (Resseguie et al 1985, Rock et al 1985, McDonough 1985).

The rationale for the use of clomiphene in the treatment of luteal phase deficiency is based on the experimental evidence from DiZerega & Hodgen (1981) that the functional capacity of the corpus luteum is dependant upon normal growth and maturation of the preovulatory follicle. Treatment with clomiphene is frequently associated with increased luteal phase progesterone concentrations (Hull et al 1982, Bohnet et al 1986), presumably secondary to the formation of more than a single corpus luteum. However, some authors regard the use of clomiphene for the treatment of CLD as inappropriate (Taymor 1986) since a proportion of patients given clomiphene for ovulatory induction will develop CLD as a result of their treatment (Garcia et al 1977, Jones et al 1970). Adjunctive hCG therapy has also been used in the treatment of threatened and recurrent abortion and CLD (reviewed by Harrison 1985). The results of the multicentre MRC trial comparing hCG with placebo are awaited.

1.3.e.ii. Polycystic ovarian syndrome.

Endocrine evaluation of patients with the polycystic ovary syndrome (PCOS) reveals a characteristic hormonal profile of raised plasma concentrations of luteinising hormone (LH) and testosterone with normal concentrations of follicle stimulating hormone (FSH) (McArthur et al 1958, Yen 1986). Patients with PCOS typically have menstrual disturbances and hirsutism, but there is a spectrum of clinical presentation (Goldzieher & Green 1962) many patients presenting with infertility (Adams et al 1985, Hull 1987). However, it is only since the advent of high resolution ultrasound imaging that an accurate estimate of the prevalence of polycystic ovaries has been possible. Adams et al (1986) described polycystic ovaries in 57% of anovulatory women and in 90% of women with hirsutism and regular ovulatory menstrual cycles. In a subsequent ultrasound study of normal female volunteers of reproductive age, PCOS was diagnosed in 23% of women who were not taking oral contraceptives and in 20% of those who were on the pill (Polson et al 1988).

These data demonstrating that the prevalence of PCOS in the general population is high and the knowledge that luteal phase defects are common

in women with the condition has led to the suggestion that PCOS may be an important cause of sporadic and recurrent abortion. Furthermore, women with PCOS tend to have a family history of relatives with PCOS (Hague et al 1986, Polson et al 1988). In a recent study of women attending a recurrent abortion clinic, ultrasound evidence of PCOS was noted in 82% of recurrent aborters compared to an incidence of 18% in parous control women (Sagle et al 1988). Furthermore, the incidence of spontaneous abortion in women with PCOS undergoing ovulatory induction therapy for infertility with clomiphene (Garcia et al 1977, Adashi et al 1979), or pulsatile LHRH is high (Adams et al 1985, Jacobs et al 1987, Homburg et al 1988, Eshel et al 1988). It has been suggested that this high incidence of abortion associated with the treatment of anovulatory PCOS may be due to impaired luteal function (Ory 1988).

An alternative explanation for these findings is that exposure of the ovaries to high LH concentrations during the phase of follicular growth may be deleterious to the developing oocyte (Jacobs et al 1987). These oocytes if fertilised may produce embryos of low viability with a higher tendency to abort. There have been several reports of high follicular LH concentrations in women with PCOS undergoing treatment with pulsatile LHRH therapy for anovulatory infertility (Abdulwahid et al 1985, Filicori et al 1988). Eshel et al (1988) suggested that this high incidence of early pregnancy failure might be correlated with hypersecretion of LH during the phase of maximum follicular growth. This association was confirmed in a subsequent report by the same group of workers (Homburg et al 1988). The serum LH levels were higher in women with polycystic ovaries who had an early pregnancy loss compared to those whose pregnancies progressed to term. Hypogonadotrophic patients on the same drug regime were noted to have lower LH concentrations and a lower incidence of spontaneous abortion. Data from IVF units is accumulating to suggest that elevated levels of LH during follicular development are associated with impaired fertilisation and reduced fecundity (Stanger & Yovich 1985, Howles et al 1986, 1987a, Macnamee et al 1987, Punnonen et al 1988).

It is possible that the endocrine disturbances found in a condition as prevalent in the general population as PCOS, may be the underlying cause of many early spontaneous abortions (Homburg & Jacobs 1989).

1.3.f. Immunological.

The fetus has earned the title of "nature's successful allograft" since it expresses paternally derived antigens inherited from the human leucocyte antigen (HLA) genes of the father and is effectively a foreign transplant in the mother's uterus. The possibility that some cases of recurrent spontaneous abortion are caused by an alteration in the normal feto-maternal relationship has received considerable attention over the last decade, since this hypothesis has important implications. An understanding of the mechanisms whereby the fetus evades rejection could provide a rationale for the treatment of previously unexplained cases of recurrent abortion and the development of contraceptive vaccination programmes. Furthermore, it would provide some valuable insights into the related areas of transplantation and tumour biology.

A review of the factors which may play a part in fetal allograft survival is needed before being able to consider immunologically mediated spontaneous abortion and its treatment.

1.3.f.i. Previous theories for the survival of the fetal allograft.

The theories first proposed by Medawar (1954) to explain fetal allograft survival have had to be modified in the light of more recent developments in reproductive immunology.

1) Antigenic immaturity of the fetus - the fertilised ovum is known to express both major and minor transplantation antigens of the histocompatibility gene complex (MHC) from early in embryogenesis (Seigler & Metzgar 1970). There is evidence that the fetus plays an active role in its own protection by developing suppressor cells capable of responding to maternal lymphocytes by the eighth week of gestation (Unander & Olding 1981). In vitro studies have shown that fetal lymphocytes can release soluble factors, possibly prostaglandins PGE1 and PGE2, that inhibit their response to adult lymphocytes (Jacoby et al 1984).

2) Immunological suppression of the mother - there is evidence that the mother can mount both a humoral and cell mediated response towards the paternally derived HLA specificities of fetal antigens. Indeed one of the oldest observations in reproductive immunology is that some pregnant women produce anti-paternal cytotoxic antibodies which apparently cause no harm to the fetus (Van Rood 1958). Maternal cellular immunity may be modified during pregnancy, but there is no convincing in-vivo evidence that it is significantly depressed (Rocklin et al 1979, Sargent et al 1988).

3) Presence of an anatomical barrier - there is good evidence to show that the placenta is not an effective immunological barrier. Transplacental haemorrhage is the principle stimulus for rhesus isoimmunisation and inevitably allows the entry of fetal leucocytes into the maternal circulation (Zipursky et al 1963) and fetal red blood cells can be detected in the maternal circulation early in pregnancy (Schroder 1975, Woodrow & Finn 1966). Trophoblast cells are shed in large quantities daily and have been reported in the maternal peripheral circulation in 80% of normal pregnant women (Goodfellow et al 1984, Covone et al 1984).

4) The uterus as an immunologically privileged site - the pregnant uterus retains the capacity of immune responsiveness during pregnancy. Extra uterine pregnancies survive and allogeneic skin grafts placed in the uterine lumen are rejected (Kirby et al 1966, Beer & Billingham 1974a, b)

1.3.f.ii. Current theories for fetal allograft survival.

The embryo does not come into direct contact with maternal tissues, since the placenta is interposed between mother and fetus. For this reason, a great deal of attention has been focused on this organ and many hypotheses have been proposed to explain how it evades immunological rejection.

Specialised characteristics of trophoblast.

The placenta contains many different cell populations, but it is the villous trophoblast lying bathed in maternal blood in the uterine sinusoids throughout pregnancy which forms the interface between fetal and maternal tissues. The outer syncytiotrophoblast and the underlying cytotrophoblast do not express Class 1 (HLA - A,B,C) or Class 11 (HLA - DR, DQ) antigens of the major histocompatibility complex (Faulk & Temple 1976, Barnstable & Bodmer 1978, McIntyre & Faulk 1979a, Johnson et al 1981a, Galbraith et al 1981). However class 1 antigens are present in villous stroma and endothelium (Faulk et al 1977) and on many forms of non-villous trophoblast cytotrophoblast (Redman et al 1984, Sunderland et al 1981, Wells et al 1984). This Class 1 antigen appears to be present in a modified form specific to trophoblast (Ellis et al 1986). Class 11 HLA positive cells have been found in villous stroma (Sutton et al 1983) and appear to be phagocytic (Redman et al 1987). These specialised characteristics may be central to the mechanism whereby trophoblast avoids or regulates immune recognition.

An alternative hypothesis has been proposed by Underwood et al (1985) who suggested that antigens are present on the syncytiotrophoblast but are constantly removed by maternal antibodies. In the mouse antigens on villous trophoblast are masked by a layer of inert sialo-mucin coating (Kirby et al 1964) but in the human placenta this sialomucin coat is incomplete (Beer & Sio 1982). The sialylation is probably responsible for the negative surface charge on villous trophoblast (Loke 1980a) which is thought to provide an anti-thrombotic lining to prevent clotting of the slow moving blood in the venous sinusoids. However there may be an immunological role for this surface characteristic of trophoblast which aids its survival (Loke 1989).

Specific antigens have been demonstrated on villous trophoblast which are not present on other fetal or adult tissues ((Whyte & Loke 1979, Ogbimi & Johnson 1981). The ability of syncytiotrophoblast villous membrane preparations to generate monoclonal antibodies reactive against trophoblast specific antigens have provided evidence that this tissue is not immunologically neutral (Johnson et al 1981b, McLaughlin et al 1982) and these antigens are present on all human trophoblast populations throughout gestation (Johnson & Molloy 1983, Bulmer et al 1984). Some of these antigens are shared with and exhibit cross-reactivity to lymphocytes, defined by Faulk et al (1978) as TA1 and TA2 and collectively known as trophoblast-lymphocyte-cross reactive (TLX) antigens (McIntyre & Faulk 1979a). These workers postulated that during normal pregnancy the maternal immune system recognised the TA2 antigen (present on peripheral lymphocytes) and produces antibodies preventing the recognition of TA1 antigen on the trophoblast. If TA2 is not recognised, this may lead to recognition of TA1 and termination of the pregnancy. Hence, maternal recognition of incompatible TLX antigens may be beneficial to pregnancy by generating "blocking" antibodies which help the survival of the fetus. Sharing of TLX antigens between couples may lead to non-recognition and failure to produce protective factors. Natural selection would favour TLX-incompatible matings thereby perpetuating genetic diversity in the population (McIntyre & Faulk 1982a, 1982b). It is this hypothesis that has led to present regimes of treating recurrent aborters by immunisation with their partner's lymphocytes and more recently with purified trophoblast plasma membranes in order to stimulate them to produce the appropriate response to the TLX antigen (see section 1.3.f.iv; p 85-90).

Immunosuppressive trophoblast products.

In addition to its normal hormonal functions in pregnancy, hCG may have a local immunoprotective role to play in pregnancy. Structurally, hCG is a sialoglycoprotein and can be demonstrated predominantly on the maternal surface of syncytiotrophoblast (Dreskin et al 1970, Loke et al 1972). The immunosuppressive action of hCG has been demonstrated in vitro (Jenkins et al 1972, Contractor & Davies 1973), but the concentrations needed are far in excess of those available in vivo (Stites et al 1979). It is not known to what extent the observed immunosuppressive properties of hCG in vitro are due to contaminants in the hormone preparations used (Siiteri & Sites 1982).

Schwangerschaftsprotein 1 (SP1) is produced by syncytiotrophoblast (Horne et al 1975) appearing in maternal serum at the time of implantation (Ahmed & Klopper 1985). Serum concentrations continue to rise to term and although SP1 is considered unlikely to provoke immune recognition by the mother, its high concentrations suggest that it might be a trophoblast signal of some importance. In vitro, SP1 will inhibit lymphocyte transformation (Cerni et al 1977), thereby preventing the production of lymphokines but only at concentrations well in excess of physiological values (Johannsen et al 1976).

Pregnancy associated plasma protein-A (PAPP-A) is produced by trophoblast and maternal endometrium and may play a local immunosuppressive role by helping to maintain the defensive fibrin barrier between trophoblast and maternal immunoreactive cells, since it appears to inhibit complement fixation (Bischof 1981) and is bound to the fibrin overlying the chorionic villi (McIntyre et al 1981).

Transferrin receptors are detectable on syncytiotrophoblast surface membranes (Galbraith et al 1980, Booth & Booth 1982). Their prime function must be the transfer of iron from mother to fetus, but it has been suggested (Faulk & Hsi 1983) that these receptors may confer a non-specific protection against infective organisms requiring iron for their metabolism, by depleting the amount of maternal transferrin available in the intervillous space.

Immunosuppressive factors produced by the pre-implantation embryo.

The pre-implantation embryo may release a variety of soluble factors that alter the maternal environment. Early pregnancy factor (EPF) is a heat stable substance found in embryo cultures from the 2-16 cell stage and in maternal circulation within 48 hours after fertilisation (Morton et al 1984).

EPF has immunosuppressive activity in vitro, binds to lymphocytes (Cocchiara et al 1986) and may play a role in suppressing maternal lymphocyte activity at the implantation site (Morton et al 1977, Smart et al 1981). The in vitro rosette inhibition assay used to identify EPF demonstrates a suppression of maternal lymphocyte activity by EPF during early pregnancy (Smart et al 1981). Spontaneous abortion is preceded by a reduction in EPF (Rolfe 1982), which supports the suggestion that reduced immunosuppression may contribute to abortion mechanisms.

It has been suggested that platelet activating factor (PAF), another pre-implantation signal derived from the fertilised ovum, may be the same as one component of the early pregnancy factor (the ovum factor) (O'Neill 1985c). Platelet activation may be caused by either activation of the coagulation cascade or as the consequence of an immune reaction. Since there is no evidence that either of these events occur within hours of fertilisation, it is presumed that the thrombocytopenia characteristic of platelet activation in early pregnancy is the result of direct action by PAF on the platelets themselves. Platelet activation follows the transfer of 2 cell mouse embryos to pseudopregnant females or the injection of culture medium in which embryos have been grown (O'Neill 1985b). A thrombocytopenic agent has been demonstrated in human embryo cultures and its presence is an indication of the viability of the pregnancy upon subsequent embryo transfer (O'Neill et al 1985).

The human zygote has been shown to produce a factor detectable in culture medium in vitro, which is directly immunosuppressive (Daya & Clark 1986). Only embryos producing suppressor activity resulted in pregnancy, suggesting that successful implantation depends upon prior maternal immunosuppression.

Maternal humoral immune responses.

As previously mentioned the production of cytotoxic antibodies to paternal HLA antigens by pregnant women apparently causes no harm to the fetus (Jensen 1964, Harris & Lordon 1976). They are detectable in some women in their first pregnancy, presumably as a result of feto-maternal bleeding (Ahrons 1971), but are believed to be significantly more common in highly multiparous women (Payne 1962, Jensen 1962, Burke & Johannsen 1974). The specificity of these antibodies may be HLA-A,B or C as well as DR (Vives et al 1976). It has been suggested that such antibodies are generated in most normal

pregnancies (Mowbray et al 1983, Tongio et al 1972, Jonker et al 1977) but are only detectable in some women because they are fixed on the fetal tissues (villous stroma) possessing the antigens against which they are directed. They may result in antigen modulation by coating antigens on the trophoblast thereby altering their immunogenicity (Underwood et al 1985). Cell-mediated responses can be blocked by antibodies present in pregnancy sera in a non-specific fashion. The ability to inhibit the MLR can be explained by the presence of anti-HLA antibodies in pregnancy sera which bind to stimulator cells (Albrechtsen et al 1977).

Non-cytotoxic antibodies directed to unidentified HLA determinants (HLA linked) are found in first trimester pregnancy sera (Pence et al 1975, Rocklin et al 1976, Stimson et al 1979) although they decline towards term (Power et al 1983a, b, Carter et al 1986). Similar antibodies have been detected in pre-transplant sera from renal transplant patients and their presence correlated with graft survival (MacLeod et al 1982, 1983). The stimulus for their production is not known, although the preliminary evidence suggests that they are neither HLA-A,B,C,DR nor TLX antibodies (Mason et al 1986).

Evidence for maternal antibodies to trophoblast has been obtained using an ELISA technique with syncytiotrophoblast membrane as the target (Davies 1985). They have been detected in pregnancy sera with maximum levels in the first trimester which gradually decline to term (Davies & Browne 1985) although this has not been confirmed by other workers (Nickson & Sutcliffe 1986). The observation of Jalali et al (1989) that all normal human placentae have IgG bound to a placental antigen suggests that there is a normal humoral response to trophoblast antigens.

Maternal cell mediated immunity.

Specific cytotoxic T lymphocytes destroy foreign cells by lysis. There are 2 stages to this response and in vitro tests have been devised that correlate with these two processes. The mixed lymphocyte reaction (MLR) measures the proliferative phase and the cell-mediated lympholysis (CML) measures the cytotoxic phase.

Using a one-way MLR to investigate immune reactivity during pregnancy many studies have shown a depressed response between maternal/paternal or maternal/fetal lymphocytes when compared with unrelated donors (Ceppellini et al 1971, Birkeland & Kristofferson 1980, Jenkins & Hancock 1972), particularly in highly parous women (Sargent et al

1987). The ratio between T and B lymphocytes is decreased during pregnancy (Rocklin et al 1979) as is the ratio between helper T cells and suppressor T cells (Beer & Quebbeman 1982). However, any depression in the lymphocyte activity occurs too late in pregnancy to offer protection against the early conceptus (Purtilo et al 1972, Garewal et al 1978).

Sensitisation to fetal (paternal) HLA has also been detected by measuring the release of a lymphokine (macrophage migration inhibition factor MIF) from maternal lymphocytes (Rocklin et al 1973, 1982) and from pooled placental antigens (Stimson et al 1979, Youtananukorn 1974). The evidence for circulating cytotoxic cells in pregnancy is conflicting. Maternal lymphocytes that are weakly cytotoxic for fetal target cells have been demonstrated in some women but are not a consistent finding in normal pregnancy (Sargent et al 1987).

Local uterine mechanisms.

The placenta can release factors that suppress lymphocyte activation. Microvillous preparations of syncytiotrophoblast and culture supernatants from placental cells non-specifically suppress mitogen responses and lymphocytes in the MLR (Rubenstein et al 1982). Suppressive activity may appear very early in gestation as human embryos have been reported to produce inhibitory factors within 24 hours of fertilisation (Daya et al 1986).

Immunosuppressive factors derived from the placenta and present in pregnancy sera have been shown to suppress lymphocyte responses non-specifically (Davies & Browne 1985). Both hCG and progesterone have been implicated since physiological levels of both hormones can suppress lymphocyte responses. The activity of progesterone is interrelated with prostaglandins which are synthesized by the placenta, amniochorion and decidua. Progesterone treated lymphocytes release a soluble factor that inhibits the production of PGF₂, thereby favouring the production of the prostaglandin PGE series, which suppress natural killer (NK) cell activity and maternal T-cell responses by switching off production of Interleukin 2 (IL2). Indeed, the inhibition of the MLR by pregnancy sera can be completely restored by adding IL2 to the cultures (Nicholas & Panayi 1985). This inhibitory activity is not found in all peripheral blood samples but is consistently found in retroplacental sera (Nicholas et al 1986). This supports the idea that the activity is derived from the placenta or decidua. It is possible that this inhibitory activity could be related to the various pregnancy specific proteins

which are known to be immunosuppressive in vitro (Nicholas & Panayi 1986).

Suppression of cell mediated responses in vitro by human decidua have been demonstrated (Daya et al 1985a). It has been proposed that suppressor activity is associated with two populations of cells, one is a large granular lymphocyte appearing in the endometrium in the luteal phase and thought to be hormone dependent (Daya et al 1985b). The second is a later phase small cell, which appears to be trophoblast dependent. These cells are reported to be absent from the decidua of women who recurrently abort (Clark et al 1987).

Immunohistochemical studies of the human decidua in early pregnancy have shown the presence of numerous cells of bone marrow origin including the "endometrial granulocyte" (Bulmer & Sunderland 1984). These cells infiltrate the decidua in the secretory phase of the menstrual cycle, just before ovulation, increase in numbers in early pregnancy and disappear after the first trimester (Bulmer et al 1987). Their presence in the latter part of the normal menstrual cycle suggests a role in preparing the maternal tissue for successful implantation and early pregnancy. It has been suggested that the endometrial granulocytes are the decidual suppressor cells but this hypothesis awaits confirmation by functional studies.

Summary.

There is evidence to suggest that the trophoblast contributes to its own survival by being relatively immunologically inert. If there is nothing for the mother to recognise as foreign, this could provide a valid mechanism for placental evasion of maternal immunological rejection. Similarly, maternal immunosuppression may be all that is required for successful placentation, although it is difficult to ignore those observations which show that the maternal immune response is not simply lower, but is actually different to the response mounted by non-pregnant women. However contemporary opinion is being swayed towards the concept that successful pregnancy requires positive maternal recognition of the fetus in order to produce a qualitative modification of her immune system that is non-destructive. For example, it is suggested that maternal recognition of trophoblast associated antigens may be beneficial to pregnancy possibly by producing blocking factors which protect the pregnancy, although what is being blocked is unclear. This hypothesis has appeal, since it provides a mechanism whereby reproduction helps to

maintain the polymorphism and genetic diversity in the population, but the direct evidence in support of this concept is not available. Immunoregulation may be mediated by non-specific suppressor factors derived from the placenta and decidua and acting at a local uterine level. At present there is no complete explanation for the tolerance of the mother to her feto-placental allograft. From the available evidence there must be several mechanisms, operative at different stages of gestation which complement each other.

1.3.f.iii. Immune characteristics of recurrent abortion.

That successful pregnancy may involve a specialised immune response unique to the feto-maternal relationship is supported by studies of women suffering recurrent spontaneous abortions.

A number of studies have reported that couples who suffer repeated abortions share more HLA antigens than do couples with normal pregnancies. It is possible that increased sharing of HLA alleles between partners could lead to the inheritance of recessive lethal genes which are incompatible with fetal development (Thomas et al 1985, Gill 1986). Detrimental effects of histocompatibility are indicated by the high pregnancy wastage in inbred rodents (Billington 1964) and decreased reproductive performance among the Huttites who share HLA- A or B (Ober 1983). A genetic mechanism is supported by the observation that the offspring of secondary recurrent aborters are more frequently premature and have a higher incidence of congenital abnormalities including Down's syndrome (Mottironi et al 1983), neural tube defects (Schacter et al 1979) acquired immunodeficiency syndromes (Pollack et al 1982) and childhood leukaemias (Von Fliedner et al 1983). However fully HLA compatible parents can have children normally (Kilpatrick 1984).

The immunological interpretation of increased HLA sharing is that compatibility results in maternal hyporesponsiveness to paternal antigens and failure to generate the appropriate protective response to maintain the pregnancy. For example a failure to produce various types of blocking antibodies have been documented among women with recurrent abortion who share HLA antigens with their partner. Taylor and Faulk (1981) suggested that couples who HLA shared also shared TLX antigens, and that the TLX compatible embryo failed to stimulate maternal blocking factors. Reports of successful pregnancies following treatment in which blocking antibody production has been induced have prompted several investigators to measure

various immune responses amongst recurrently aborting women in order to identify those who may fall into this potentially remediable category.

HLA typing.

Komlos et al (1977 and 1979) found increased HLA sharing in 70% of couples with a history of one abortion and 77% of couples with repeated abortions, compared to a 38% incidence in a control group with two or more normal pregnancies. Schacter et al (1979) noted that couples with three or more normal pregnancies never shared two or more HLA antigens, but when there had been two or more first trimester abortions the incidence was 31% (4 out of 13 couples). Increased sharing of HLA-A, but not B antigens (Gerencer et al 1979), HLA- A,B and DR antigens (Unander & Olding 1983, Caulam et al 1987) and HLA-A,B,C and DR antigens (Beer et al 1985, Reznikoff-Etievant et al 1987) amongst recurrent aborters have all been reported.

Contradictory evidence has been provided by other studies in which increased HLA sharing was either not observed (Caudle et al 1983, Oksenberg et al 1984, Mowbray et al 1983, Johnson et al 1984), or was weak and inconsistent (Gill et al 1983, Thomas et al 1985). However McIntyre et al (1984) suggested that discrepancies in these results could be due to a failure to distinguish between two differing groups of abortion prone couples. They reported HLA sharing to be more common in primary aborters than secondary aborters and controls (McIntyre et al 1986, Caulam et al 1987), although in a further study (Hofmeyer 1987) secondary aborters were shown to have a higher incidence of sharing. In a recent report of recurrently aborting couples some of whom had been specifically referred because of increased HLA sharing (Smith & Cowchock 1988), sharing of two or more HLA antigens was not significantly higher than the incidence in the control populations reported by Gill (1986) and Thomas et al (1985).

Although HLA sharing was one of the original indications for immune treatment of recurrent spontaneous abortion, most workers have abandoned this test as a criterion for treatment. The increased incidence of genes HLA-A9 (Gerencer et al 1978, 1979) and HLA-Bw35 (Komlos et al 1977) and B8-DR3 (Marelli et al 1986) in patients with recurrent abortion and with other types of auto-immune disease has not been explained. It has not been possible to correlate materno-fetal histocompatibility to pregnancy outcome (Jazwinska et al 1987) and the hypothesis of increased HLA sharing has been reviewed and challenged (Adinolfi 1986). In a recent study by Christiansen et al (1989) HLA

sharing among recurrently aborting couples with and without autoimmune aberrations was not different.

Leucocytotoxic antibodies.

The presence of serum anti-paternal cytotoxic antibody (APCA) provides evidence of maternal immunological recognition of the fetus. In women with recurrent abortion a significantly lower incidence of this antibody is reported. When all women with a history of recurrent pregnancy losses are considered together, the incidence of detectable antibody is between 12% to 20% (McIntyre et al 1986, Johnson et al 1985, Beer et al 1985, Mowbray et al 1985, Smith & Cowchock 1988). However there appear to be marked differences in the incidence between primary and secondary aborters. Amongst 209 couples investigated Mowbray (1985) found the frequency of positive APCA results in primary recurrent aborters to be only 9%, compared to 14% and 38% in women with one or two live births in addition to their three consecutive losses. By comparison, the incidence among women with successful pregnancies only was 85% (Mowbray et al 1983). A strong correlation between positive APCA status and positive MLR blocking was also noted and led these workers to conclude that the absence of APCA from the serum of recurrent aborters is a useful marker with which to identify women who have failed to mount an appropriate immune response towards repeated pregnancies. Similar overlap between APCA and MLR testing have been reported by Hofmeyer et al (1987) and Smith and Cowchock (1988)

McIntyre et al (1986) suggested that primary and secondary recurrent aborters represent two distinct populations who can be discerned by immunological testing. The primary aborters do not manifest anti-paternal immunity (incidence of APCA of 1%), they share HLA antigens with their partners and respond poorly in MLR testing. Secondary aborters do not share HLA antigens, exhibit both high frequencies (94%) and high titres of APCA which persist between pregnancies, and have a serum factor blocking the MLR which may represent an inappropriate response to TLX antigens. The differences in response between the two groups strengthens the idea that both inadequate or inappropriately vigorous maternal anti-paternal immunity can cause repeated abortions.

Blocking antibodies.

Antibodies which can inhibit the mixed lymphocyte reaction (MLR) during (Rocklin et al 1982) and after pregnancy (Gatti et al 1973) have been reported as absent or present in low titres in the serum of women with recurrent abortion (Rocklin et al 1976, Stimson et al 1979, Takeuchi 1980, Power et al 1983, Fizet & Bousquet 1983). Blocking factors detectable in plasma but not in the sera from women with recurrent abortion have also been reported (McIntyre & Faulk 1983). Antibodies with specificities other than for paternal HLA may be implicated. Anti-idiotypic antibodies that bind to the maternal T-cell receptors for paternal HLA types are found in the sera of parous women (Singal et al 1984, Sucia-Foca et al 1983).

Several groups have noted decreased reactivity of maternal to paternal cells in the one-way MLR compared with the response to a third party control (Beer et al 1981, Beer 1983, Lauritsen et al 1976, McIntyre & Faulk 1983, Unander & Olding 1983, Unander & Lindholm 1986). Blocking capacity appears to be a useful screening test for assessing women with recurrent abortion and their suitability for immune treatment. The finding of strong MLR blocking activity in a woman's serum, most probably secondary to underlying autoimmune disease (Unander et al 1985), is considered an absolute contraindication to therapy by some workers (Unander & Lindholm 1986).

1.3.f.iv. Immunisation treatment programmes.

In 1981 Taylor and Faulk published the first report of donor leucocyte transfusion treatment in three women with idiopathic recurrent spontaneous abortions. These three women all shared more than 2 HLA antigens with their partners, which the authors suggested might reflect that they also shared trophoblast antigens. They were transfused at three weekly intervals with leucocyte enriched plasma from 16 different erythrocyte-compatible donors and delivered healthy babies at term. It was suggested that the TLX antigens on the transfused leucocytes stimulated maternal recognition of the embryo and the production of anti-TLX blocking antibodies, which protected the pregnancies.

Further reports of successful pregnancy outcome after infusions of paternal leucocytes (Beer et al 1981, Mowbray et al 1983), donor leucocytes (Beer et al 1983) and leucocyte rich erythrocyte concentrates (Unander et al 1985) followed. Irrespective of the dosage (single or multiple), route of

administration (intravenous, intradermal and subcutaneous) or the source of cells (paternal or third party, whole blood, blood fractions) success rates of 75% to 85% were obtained, creating public demand for treatment. The immunisation regimes that have developed since these first reports are summarised in Table 1.5.

Table 1.5

IMMUNISATION TREATMENT RESULTS

Author	Source	Dose	Patients treated	Pregnancies	Live births	Success rate %
Taylor et al 1985	Donors	Multiple	28	21	17	81
Mowbray et al 1985	Paternal	1	105	22	17	77
	Maternal	1		27	10	37
Mowbray et al 1987	Paternal	Before pregnancy	145	77	59	77
		During pregnancy	76	75	53	71
Beer et al 1985	Paternal	2	77	39	28	72
	None		131	44	28	64
Beer 1988	Paternal	2	121	121	100	83
	Third party		21	21	15	72
	None		51	51	13	25
Unander et al 1986	Third party	3	49	25	24	96
McIntyre et al 1986	Donors	Multiple	33	26	23	88
Takakuwa 1986	Paternal	3	10	7	5	71
Carp et al 1988	Paternal	3+	70	34	27	79
Cowchock 1988	Paternal	1		50	27	54
Alexander 1988	Paternal	Multiple	59	26	20	77
Reznikoff -	Paternal	Multiple	34	34	29	85
Etievant et al 1988	None			28	10	36
Johnson et al 1988	Placental eluates		21	21	16	76

The results of the only controlled double blind randomised trial conducted by Mowbray et al (1985) are of particular importance. Patients were

admitted to the study after three consecutive abortions with the same partner if they had no detectable APCA and no other documented cause for their spontaneous abortions. The treatment group received lymphocytes prepared from 500mls of their partners blood. Immunisation was performed intravenously (3mls), intradermally at 2 sites (1ml) and subcutaneously at 2 sites (1ml) on a single occasion. The women in the control group were injected with their own lymphocytes. Seventeen of the 22 women (77%) given paternal cells delivered live children, compared with 10 of 27 (37%) given their own cells ($p=0.01$). No patient side effects occurred and no serious congenital abnormalities in the babies were detected. Successful pregnancy outcome correlated strongly with the production of APCA. Of the women immunised with paternal cells 76% made APCA and 85% of these delivered liveborn children. No other pre- or post immunisation assays were performed, so it is unknown whether other antibody or cell-mediated responses were initiated.

A further report by the same authors in 1987 confirmed the efficacy of this form of treatment and included an additional group of women who underwent immunisation early in pregnancy (Table 1.5). Only 24% of those women immunised during early pregnancy became APCA positive compared with a 65% seroconversion rate in women treated before pregnancy. The enlarged series demonstrated that women who developed APCA after treatment were protected in pregnancies occurring up to 12 months after treatment whereas those who remained APCA negative and became pregnant more than 80 days after treatment were likely to miscarry. Booster injections for this latter group were suggested.

The results published by Beer et al (1985) included 39 women without blocking antibodies who received two doses of subcutaneous paternal lymphocytes, of whom 29 (72%) delivered a normal baby. The production of blocking antibody following treatment correlated with successful outcome. A control group of 44 women received "general medical treatment" and 64% of this group had a successful pregnancy. Subsequent reports included larger numbers (Beer 1988) and the introduction of third party lymphocyte infusions for women with treatment failures after paternal infusions (see Table 1.5). MLR blocking was low or absent in the failed "paternal" treatment group, but following third party immunisation MLR blocking activity to paternal and third party stimulator cells was strong. The presence of APCA and the absence of MLR blocking was predictive of a further pregnancy loss following paternal

immunisation and the authors suggested that this correlates with a lack of anti-idiotypic receptors to maternal T cell receptors. These studies concluded that reproductive outcome can be predicted by the MLR in vitro response following paternal immunisation. Third party therapy was successful for failed paternal immunisation and those who experienced intrauterine growth retardation after paternal immunisation (15%), because this subgroup of patients probably share the same TLX antigens. Third party immunisation induced measurable immune responses in these patients which were associated with improved outcome in the subsequent pregnancy.

Unander and Lindholm (1986) studied 49 recurrent aborters and found no blocking antibody (as measured by MLR hyporesponsiveness) in 38 women who received 3 infusions of leucocyte rich erythrocyte concentrates at 2 monthly intervals. All 38 of these women developed blocking antibody after treatment and of the 25 subsequent pregnancies only 1 aborted. The 11 women in the study with pre-treatment blocking antibody developed increased activity after treatment. Five subsequent pregnancies all aborted. These 5 women were all subsequently identified as having serological signs of autoimmune disease. McIntyre et al (1986) treated 33 MLR negative primary aborters with multiple infusions of donor white cells and reported a success rate of 88%. Secondary aborters were not immunised since these workers consider that the immune mechanisms responsible for abortion in these patients are unlikely to be helped by this form of treatment (see section ii b). Indeed the 8 secondary aborters immunised by Smith & Cowchock (1988) had a low pregnancy success rate of only 25%.

Repeated injections of paternal cells until patients become APCA positive are suggested by Reznikoff-Etievant et al (1985, 1988) who have found a strong association between positive APCA status and successful pregnancy outcome, although they point out that these antibodies may be an indirect index of effective immunisation. To support this argument they reported successful pregnancy outcomes in 93% (14/15) women who were not immunised but were APCA positive when first investigated, comparing them to an untreated group of APCA negative women of whom only 36% (10/28) had subsequent live births. In a recent report Smith and Cowchock (1988) found no association between successful outcome and positive APCA or MLR blocking status after immunisation. Indeed their results suggested a negative correlation with APCA development.

The pilot study of immunisation with purified trophoblast vesicles from pooled donor placentae reported by Johnson et al (1988) would seem to be a more physiological approach. The results of their double blind placebo controlled trial are awaited.

Concern has been expressed by some clinicians about the potential risks involved in immunisation treatment. Recessive lethal genes may be the cause of some cases of recurrent abortion as discussed earlier (Gill et al 1986) and deliberate maternal immunisation may affect the development of the fetus and placenta resulting in a fetal wastage syndrome not unlike graft versus host disease (Adinolfi 1986). In one study (Menge & Beer 1985) severe growth retardation was reported in 11% (3/28) of babies born after paternal immunisation and another infant was born alive with trisomy 13, while the infants born to "control" mothers exhibited no such abnormalities. An isolated case of neonatal alloimmune thrombocytopenia possibly related to anti-PlA¹ antibodies boosted in titre by immunisation treatment has also been reported (Smith & Cowchock 1988).

The incidence of congenital abnormalities and low birth weight noted in the 150 babies born following immunisation treatment reported by Mowbray et al (1987) was not greater than that expected for the general population. However, since one of the established risk factors for intrauterine growth retardation is a history of abortion or intrauterine death (Wennergren et al 1982, Reginald et al 1987) it would not be surprising to find an increased incidence of IUGR among treated or untreated recurrent aborters. At the present time there is insufficient data available on the long term effects of immunisation, since the oldest children are not yet adult. One of the conclusions of the Royal College of Obstetricians and Gynaecologists study group on early pregnancy loss was that a register should be started to record all children born to recurrent aborting couples (Mowbray 1988).

A variety of maternal side effects have been reported following immunisation treatment, including red cell sensitisation, anaphylaxis, serum sickness and flu-like symptoms (Hofmeyer et al 1987). Theoretically, the risks are greater when multiple blood transfusions from pooled donors are used. Another hazard is the possible transmission of hepatitis, toxoplasmosis, cytomegalovirus, HIV and other infectious agents. In addition, patients treated in this way may be at risk of developing or of exacerbating existing subclinical auto-immune disease (Marelli et al 1986, Christiansen et al 1988). It has also been suggested that immunisation treatment may marginally impair fertility

since the pregnancy rate in the untreated patients in Mowbray's series (1985) was 25% higher than that noted in the immunised group (Hofmeyer et al 1987).

Several authors have suggested that the success of immunisation treatment may be largely due to a placebo effect (Adinolphi 1986, Moloney et al 1989). This question is difficult to address since couples suffering recurrent episodes of spontaneous abortion are a desperate group who demand treatment and are reluctant to participate in placebo controlled trials. Since the risks associated with this form of treatment may prove to be considerable and the acceptance of this form of therapy is based on the results of a single controlled trial, several clinicians feel that immunisation treatment should only be considered for patients participating in randomised double-blind controlled trials (Sargent et al 1988, Alexander et al 1988).

1.3.f.v. Blood group incompatibilities.

Incompatibility between mother and fetus in the ABO and the P blood group systems may be a cause of spontaneous abortion. In 1925, Hirszfeld and Zborowski noted a deficiency of offspring with blood group A in matings of an A father and an O mother. There is some evidence that this phenomenon is due to a higher frequency of both infertility and spontaneous abortion in ABO incompatible couples (Matsunago & Ito 1958) with fetal loss rates of up to 18% (Lauritsen et al 1975). Group O mothers are more frequently affected because they are incompatible to all fetuses except those who are also Group O, and are more likely to develop IgG antibodies when sensitised by fetal incompatible erythrocytes. Blood group typing of abortuses has revealed a higher incidence of A and B compared to O abortuses among chromosomally normal abortions (Krieg & Kasper 1967, Lauritsen et al 1975) and a significantly higher frequency of serological incompatibility between mother and fetus than would be expected on the basis of ABO frequencies in the population (Takano & Miller 1972). These findings have led to the suggestion that fetuses which are both karyotypically abnormal and ABO incompatible with their mother are aborted so early in pregnancy that they are rarely included in studied samples.

The mechanism whereby ABO incompatibility may cause abortion is not known, but since ABO antigens are present on the surface of most fetal tissues Szulman (1973) has suggested that any immunological interaction could disrupt organogenesis in the whole fetus and thus cause abortion. Mourant et al (1978) have proposed that amongst couples with repeated

abortions that there will be an excess of group O mothers, group A or B fathers and fetuses of group A and B. However, the blood groups of aborted fetuses are rarely recorded and therefore direct evidence that ABO incompatible couples are at greater risk of recurrent abortion is lacking.

Within the P blood group system a causal relationship has been claimed between the presence of maternal antibody anti-PP₁P^k (anti-Tj^a) and recurrent abortion (Levine & Koch 1954, Weiss et al 1975). The pathogenesis is unknown but anti PP₁P^k is an IgG antibody and therefore able to cross the placenta.

An increased frequency of spontaneous abortion has been reported in matings between Rhesus-D positive fathers and Rhesus D negative mothers (Cohen 1970) and within the Lewis system (Elbel et al 1952). However Lauritsen et al (1976a) concluded that incompatibility between parents of Rhesus, Lutheran, MN, S, Lewis, Kell, P and Duffy systems was not significant in the aetiology of early spontaneous abortions.

1.3.f.vi. Anti sperm antibodies.

An increased abortion rate has been reported in women with sperm-agglutinating antibodies (Jones 1976) and infertility (Menge et al 1982). However the majority of studies have been centred on patients undergoing infertility investigations and normal control groups are few. Among 109 couples referred for unexplained infertility Witkin & David (1988) reported a spontaneous abortion incidence of 44% in women with IgA and IgM anti-sperm antibodies. In this study the presence of anti-sperm IgA antibodies in female sera was associated with spontaneous abortion but not with a failure to conceive, suggesting that this sperm antibody isotype maybe a marker for increased susceptibility to spontaneous abortion. The mechanism of action is unclear, but may involve the cross-reactivity of sperm membrane antigens with epitopes common to lymphocytes and trophoblastic tissue (Menge & Fleming 1978, Mathur et al 1980).

1.4. Other variables affecting the incidence of abortion.

1.4.a. Infertility treatment.

The first suggestions that the risk of spontaneous abortion is higher than normal in patients with infertility who subsequently conceive predate modern methods for treating infertility. Moreover the frequencies quoted

were based on spontaneous abortions diagnosed by clinical presentation alone. In a prospective study of 1,497 pregnancies (Tietze et al 1950), a long preconception interval was noted to be a significant risk factor for spontaneous abortion and the authors postulated that "the same pathological conditions which delayed conception were responsible for the abortion". The incidence of abortion among primary infertility patients receiving no specific treatment was recorded as 19.3% (Bender 1952) compared to 11% in spontaneous primigravid conceptions of comparable age (Reed & Kelly 1958). Reports of higher abortion rates began to emerge following the introduction of treatment programmes for infertile couples. Weir and Hendricks (1969) reported an overall abortion incidence of 31% among 423 couples receiving varied treatments in their clinic and with the availability of β HCG testing to detect early implantation losses in addition to clinical pregnancy losses, Chartier et al (1979) found that 37% of "assisted" conceptions ended in spontaneous abortion before 12 weeks gestation.

More recently, the introduction of in vitro fertilisation techniques and sophisticated in vivo gonadotrophin regimes for the treatment of infertility, have produced a vast amount of literature describing methods in which pregnancy rates can be maximised with each of the available treatment options. Some of the data is difficult to interpret due to competitive pressures which encourage reports of successful conceptions as opposed to successful pregnancy outcomes.

Overall, the incidence of spontaneous abortion ranges widely from 12% to 45%, since the relative risks of the various methods aimed at achieving conception appear to be different (reviewed by Jansen 1982a). Much of the detail is beyond the scope of this thesis, but a brief summary is included here, since an improved understanding of the mechanisms whereby these treatment regimes result in success or failure are beginning to shed some light upon the physiology of normal implantation, early pregnancy and spontaneous abortion.

1.4.a.i. Ovulation induction.

Clomiphene citrate is the most commonly used drug for the induction of ovulation, and the frequency of spontaneous abortion associated with its usage ranges from 10- 27% (Garcia et al 1977, Correy et al 1982, Adashi et al 1979, Jansen 1982). Several possible mechanisms have been implicated. Abnormalities in the karyotypes of abortuses are reported more commonly

(Boué & Boué 1973c), suggesting that clomiphene may increase the risk of chromosomally unbalanced oocytes (Wramsby et al 1987). Maturing follicles with gross morphological abnormalities have been described (Jones et al 1970) in particular neural tube defects (Dyson & Kohler 1973, Sandler 1973) Multiple pregnancy rates are also higher than normal (Asch & Grenblatt 1976).

Although plasma progesterone levels after clomiphene treatment are usually normal or increased (Hull et al 1982, Bohnet 1986), endometrial luteal phase biopsies show defective progesterone action in up to 50% of cycles (Garcia et al 1977), most probably due to clomiphene's paradoxical anti-oestrogenic actions at the endometrial level in reducing the number of oestrogen primed endometrial progesterone receptors (Fritz et al 1987). Lower than usual abortion rates after clomiphene have been reported in patients receiving supplemental oestrogen therapy in the late follicular phase (Poliak 1973), or luteal phase progesterone (Garcia et al 1977, Wentz 1984).

Clomiphene therapy is frequently used as a first line treatment for PCOS, a syndrome characterised by high circulating Luteinising Hormone (LH) levels. Quigley et al (1984) have demonstrated a dose dependant increase in LH secretion after clomiphene administration. An increasing amount of evidence is accumulating to suggest that elevated levels of LH during follicular development are associated with impaired fertilisation and pregnancy loss (Chapter 5; p 178).

The incidence of abortion following gonadotrophin therapy has been estimated at 23% (reviewed by Jansen 1982a). The figure seems to depend upon patient selection, for example the abortion rate among hypogonadotrophic hypoeutrogenic patients is lower (23%) compared to the incidence in women with normal gonadotrophin levels and endogenous oestrogen activity (42%) (Lunenfeld 1979). Evans and Townsend (1976) have suggested that the higher incidence of abortion in patients with endogenous gonadotrophin activity could be due to 1) a premature endogenous LH surge, 2) follicular development already being underway at the start of therapy, making FSH requirements unpredictable and 3) preselection of patients with refractory ovulatory dysfunction who have failed to respond to clomiphene.

The relative concentration of FSH and LH in the preparation used may be important, particularly in view of the recent association between high tonic LH levels in the follicular phase of the cycle and poor pregnancy outcome. The incidence of abortion in PCOS patients treated with pure FSH is lower, which may be due to the suppressive effect of these FSH preparations on serum LH

levels (Jones et al 1985). Couzinet et al (1988) have demonstrated significantly higher levels of LH in women with hypopituitarism treated with hMG preparations (FSH:LH) compared to those on pure FSH and Jeffcoate (1985) noted that in IVF patients late follicular LH concentrations were significantly raised on combined clomiphene/hMG therapy, compared to patients having hMG alone.

Pulsatile LHRH therapy has been used to induce ovulation in women with polycystic ovaries who are resistant to clomiphene. In contrast to the good treatment results obtained in hypogonadotropic patients (Armar et al 1986), PCOS patients respond poorly to treatment and have an increased rate of early pregnancy loss (Homburg 1988), further implicating the adverse effect of high LH levels upon pregnancy outcome. It has been suggested that the use of GnRH analogue agonists to induce a hypogonadotropic state in PCOS and IVF patients may improve pregnancy outcome. Many studies have reported high success rates (in terms of pregnancies conceived) in IVF patients with previously abnormal levels of LH or those who responded poorly to clomiphene/hMG regimes (Macnamee et al 1987, Rutherford et al 1988).

The spontaneous abortion rate after treatment of hyperprolactinaemia with bromocryptine is low, averaging 12% (Pepperrell et al 1977, Griffith et al 1978, Spark 1979, Jansen 1982a), most probably because successful ovulation induction with bromocryptine for hyperprolactinaemia is accompanied by gonadotrophin levels that approximate to normality more closely than after exogenous gonadotrophins or clomiphene (Pepperrell 1977). A higher abortion incidence is reported in patients receiving clomiphene simultaneously (Thorner et al 1979).

1.4.a.ii. Artificial Insemination.

Couples in whom infertility results solely from azoospermia or oligospermia and who are treated with artificial insemination by donor have a low reported incidence of spontaneous abortion averaging 15% (Behrman 1979, Jansen 1982a, Yovich & Matson 1988). By contrast the quoted rates for artificial insemination by husband (AIH) are higher averaging 24%. Two series have considered abortion incidence with and without clomiphene to regulate insemination cycles and reported a higher abortion rate with clomiphene (Corson 1980).

There is no conclusive evidence that quantitative and morphological qualities of semen are related to abortion. Polyspermy, defined as a

concentration of 200-250 million sperm per millilitre has been blamed (Homomai et al 1980). Glazerman et al (1982) reported an 4% incidence of polyspermy among infertile couples and an abortion rate of 25%, but other studies have failed to find such an association (Rehan et al 1975). Similarly, oligospermia does not appear to carry an increased risk of spontaneous abortion (Joel 1966, Rehan et al 1975). However, since the prevalence of chromosomal abnormalities among male infertility patients is over 2% (Chandley et al 1975) and men carrying a balanced translocation more frequently have abnormal semen, it is difficult to know which factor is the cause and which the effect.

1.4.a.iii. IVF.

The incidence of clinically recognised spontaneous abortion following in vitro fertilisation and embryo transfer has been variably reported, figures ranging from 15% to 45% (Frydman et al 1981, Jansen 1982b, Edwards & Steptoe 1983, Andrews et al 1986, Sher et al 1986, Ben-Rafael 1988, Yovich & Matson 1988). The obstetric outcome of 1176 IVF pregnancies were pooled in the collaborative FIVNAT study (French National IVF Register) reported by Mouzon et al (1988) which concluded that the incidence of clinical spontaneous abortion was 18 - 25%. Since IVF does not increase the rate of chromosomally abnormal abortuses when compared with in-vivo fertilisation (Plachot 1989), the increased incidence of early fetal loss after IVF must be attributable to other factors. Heightened surveillance in these patients may account for some of this observed increase in early pregnancy loss (Ben-Rafael et al 1988, Mausher & Garcia 1986) but does not explain it all (Yovich & Matson 1988, Jansen 1982a).

Edwards et al (1984) considered that implantation is impaired in older women. A maternal age of over 40 years and multiple pre-embryo transfer are the two most significant factors now generally accepted to adversely affect the incidence of spontaneous abortion (Kerin et al 1984, Fishel et al 1984, Romeu et al 1987). Careful patient selection and refinements in ovulatory induction regimes and laboratory techniques most probably account for the reduction in the abortion rate seen in those centres that have acquired experience with these assisted conception techniques (Yovich & Matson 1987).

1.4.b. Menarche.

A relationship between early age at menarche and an increased risk of spontaneous abortion, evident in a woman's first four pregnancies has been reported (Liestöl.1980). Since early menarche is associated with a high incidence of induced abortion in young women (Köller & Eikhorn 1977), and age at menarche is determined by so many environmental factors these results are difficult to interpret. Casagrande et al (1982) were only able to confirm Liestöl's findings among first pregnancies. Nonetheless this finding may still prove to be important since both early age at menarche and first trimester spontaneous or induced abortion before the first full-term delivery increases a woman's risk of breast cancer (Pike et al 1981).

1.4.c. Environmental and occupational hazards.

Although spontaneous abortion is one of the adverse reproductive outcomes expected after exposure to potentially teratogenic or mutagenic agents, epidemiological evidence of such an association is sparse (Bloom 1981). Numerous drugs and environmental chemicals have been shown to cause chromosomal damage in in vitro animal studies, but data directly implicating an effect on the human species are scarce and most handbooks on toxicity and hazards of chemicals do not refer to pregnancy. Environmental exposures which recur during a couple's reproductive life span may contribute to the aetiology of recurrent abortion. Maternal smoking and alcohol consumption are discussed in more detail below (1.4 d; p 98).

1.4.c.i. Occupational factors.

The incidence of spontaneous abortion in female agricultural workers (Hemminki et al 1980a) and hospital laboratory workers (Strandberg et al 1978) has been reported as higher than that of other types of workers. However in studies of this sort, careful correction for confounding variables is required. For example, the difference in abortion incidence noted between women working in the metallurgical area and clerical workers in a Swedish steelworks was later attributed to differences in age distribution and smoking habits (Kölmodin-Hedman et al 1982). Workers exposed to vinyl chloride have been found to have a significantly increased frequency of chromosome aberrations in their lymphocytes than did controls (Purchase et al 1975). Fetal loss was higher among wives of exposed workers, presumed secondary to male germ cell damage, although no data on the the products of conception

were obtained and the wives were not interviewed (Infante et al 1976). Studies of the incidence of abortion in the wives of workers exposed to pesticides have been reported as increased (Kharazzi et al 1980) and normal (Smith et al 1982). The association between lead poisoning and abortion (Rom 1976) has been refuted (Friberg et al 1979).

1.4.c.ii. Anaesthetic gases.

Following the report of a higher frequency of spontaneous abortion among operating theatre workers (Vaisman 1967), several large epidemiological studies confirmed the higher incidence of abortions among female anaesthetists and theatre nurses (Askrog & Harvold 1970, Cohen et al 1971, Knill-Jones et al 1972). Among female dentists and nurses, the frequency of abortion could be correlated to heavy, moderate and non-users of anaesthetic gases (Nixon et al 1979, Cohen et al 1980). However, it has been suggested that these studies suffered from reporting bias (Spence 1981) since there is a tendency for the non-exposed group to under-report abortions and the exposed group to recall all miscarriages (Axelsson & Rylander 1982) and the association remains presumptive (Ericson & Källén 1985, Hemminki et al 1985, Tannenbaum & Goldberg 1985). Pharoah et al (1977) found that women who held anaesthetic appointments at the time of conception had the same incidence of spontaneous abortions as other groups of doctors. However, the anaesthetists tended to have babies of comparatively low birth weight.

1.4.c.iii. Chemical wastes and pollutants.

There is no convincing evidence that industrial accidents, the dumping of waste or pesticide spraying increases the incidence of spontaneous abortion in the population, although anecdotal reports have implicated several substances and incidents. Frequently the finding of an increased incidence of abortion in these circumstances does not remain statistically significantly different from control subjects after confounding variables such as age, socio-economic status, alcohol and smoking habits have been controlled for.

1.4.c.iv. Radiation.

Radiation is known to induce chromosome damage in human somatic cells (Evans & O'Riordan 1975). There is also some evidence suggesting that maternal irradiation may cause abnormal gametes producing chromosomally abnormal offspring. Alberman et al (1972) found that mothers of abortuses with abnormal karyotypes (mostly trisomies and triploidies) had received

higher doses of radiation than both mothers of abortuses with normal karyotypes and those who had liveborn infants. The significant increase in the number of children with trisomy 21 amongst mothers who have received pelvic irradiation, compared to age-matched controls (Uchida et al 1968, Alberman et al 1972), has led to the conclusion that even low doses of irradiation during the reproductive period increases the risk of future conceptions with abnormal chromosome complements. Data suggesting that the risk of abortion in physicians working with ionising radiation increases with increasing parity (Freire-Maia 1970) have not been substantiated.

The increasing use of computers and word processors has led to widespread anxieties concerning the possible harmful effects of video display units (VDU) on pregnancy outcome following published reports of clusters of pregnancy failures in such workers (reviewed by Purdham 1984, Denning 1985). The more recent larger-scale studies which have corrected for confounding variables such as maternal age, alcohol, smoking and irregular shift work have not demonstrated a statistically significant association between VDU use and spontaneous abortion (Kurppa et al 1985, Westerholme & Ericson 1986) although stress associated with the job and operator posture have been implicated (Mackay 1987). At present it seems reasonable to conclude that a pregnancy is not harmed by using a VDU and that statements to the contrary are not soundly based (Blackwell & Chang 1988).

1.4.d. Alcohol and smoking.

The fetal alcohol syndrome has been well documented in the children of alcoholic mothers (Jones et al 1974, Clarren & Smith 1978), and moderate drinking is associated with growth retardation and fetal abnormalities (Little 1977, Hanson et al 1978), suggesting that alcohol has both a toxic and teratogenic effect on the fetus. In a prospective study by Harlap & Schiono (1980b) moderate quantities of alcohol increased significantly the risk of second trimester spontaneous abortion. The effect appeared to be dose related, since one or two drinks daily doubled the risk of abortion and larger quantities increased the risk still further. The increased risk could not be explained by age, parity, race, marital status, smoking or the number of previous spontaneous or induced abortions. Unfortunately the study design prevented the authors from ascertaining with certainty whether alcohol had the same effect in the first trimester. Nonetheless, an editorial (Lancet 1980) published

in the same journal issue strongly advocated that women should be counselled that avoiding periconceptual alcohol intake in small regular or large occasional amounts may prevent spontaneous abortion.

In a second case controlled study (Kline et al 1980) the dose dependant effect was confirmed, but the minimal threshold for producing an abortion was one ounce of alcohol twice weekly. These authors suggested that spontaneous abortion is the most likely outcome of regular alcohol consumption, and that the infant with congenital malformations or fetal alcohol syndrome should be considered as " a rare survivor among conceptions heavily exposed to alcohol" (Stein et al 1975). Given these findings, it is not surprising that a higher incidence of recurrent spontaneous abortion has been noted among women who drink regularly and heavily (Sokol et al 1980).

Women who smoke cigarettes regularly during pregnancy run a greater risk of spontaneous abortion than non-smokers (Kullander & Källén 1971, Harlap & Davies 1975, Murphy & Mulcahy 1978, Hemminki et al 1983, Risch et al 1988, Kline et al 1977). The association between smoking and spontaneous abortion becomes stronger (higher odds ratios) when the analyses are adjusted for maternal age, gravidity and previous miscarriages (Kline et al 1977, Himmelberger et al 1978).

Kline et al (1977) suggested that the two fold increase in risk of abortion might reflect the same mechanisms that lead to low birth weight in the infants of women who smoke, namely reduced food intake, poor maternal weight gain (Rush 1974, Davies et al 1976) and fetal anoxia (Longo 1976). They argued that it was unlikely that smoking in pregnancy is teratogenic since the adverse effect is mainly found in chromosomally normal abortions (Boue et al 1975a, Alberman et al 1976, Warburton et al 1979, Kline et al 1980). However the variables confounding this analysis are complex. The association of smoking and ex-smoking with younger age at menopause suggests that smoking may speed up biological ageing (Baron 1984), and could explain the increased risk of trisomic abortion in older women who have smoked in the past. However, this theory fails to explain the suggested "protective " effect of smoking for trisomic abortion in young women or the slightly decreased risk of trisomy 21 at birth to smokers of all ages (Kline et al 1983).

Alcohol use tends to be correlated with smoking and most studies that have found a strong association between smoking and spontaneous abortion have not taken into account the effects of drinking. In a prospective study

designed to assess both smoking and drinking habits in pregnancy independantly, the effect of alcohol on the incidence of spontaneous abortion was much stronger than smoking (Harlap & Schiono 1980). When analysed independantly, smoking showed a weak and inconsistent adverse effect on the risk of abortion.

1.4.e. Surgical procedures during pregnancy.

Surgery performed during early pregnancy has been implicated as a factor that may increase the incidence of spontaneous abortion. Since halothane anaesthesia has been shown to interfere with DNA synthesis (Pederson & Finster 1979) adverse effects are thought to be more likely during the first trimester of pregnancy and most clinicians advocate delaying abdominal surgery until the second trimester of pregnancy wherever possible. Saunders and Milton (1973) reported a fetal loss rate of 23% after surgery in early pregnancy, mostly cases of ovarian cystectomy and appendicectomy. One large retrospective study suggested that surgery was associated with a slightly elevated risk of abortion when undertaken during the first trimester and that the risk was five times higher in the second trimester (Brodsky et al 1980). However these results have been questioned since they are susceptible to recall and memory bias and no data on the type of operation or anaesthesia used were given.

1.4.e.i. Amniocentesis.

Transabdominal sampling of amniotic fluid was first reported in the 19th century (Prochownik 1877), but it was not until the 1960's that it became possible to culture fetal desquamated cells in vitro for the diagnosis of chromosomal abnormalities in high risk pregnancies (Steele & Breg 1966, Jacobsen & Barker 1967). Since then, amniocentesis has become part of standard obstetric practice and 2-3% of all continuing pregnancies are now investigated by mid-trimester amniocentesis (Turnbull & Mackenzie 1983, McNay & Whitfield 1984), the most common indication being maternal age of over 35 years. In the UK, over 17,000 cases are performed annually and the technique is available in most obstetric units (MacLachlan et al 1989).

The NICHD study (USA National Registry for Amniocentesis 1976) was the first to confirm that the frequency of spontaneous abortion is not significantly higher following amniocentesis, the incidence of second trimester pregnancy loss was 2.3% among amniocentesis patients compared to

2.1% in the controls. In the Canadian study (Simpson et al 1976) there was a reduction in the number of spontaneous abortions in the amniocentesis group. In a series of 3000 patients in San Francisco (Golbus et al 1979) and 1000 patients in South Carolina (Young et al 1983) the abortion rates after amniocentesis were 1.5% and 1.0% respectively. In the Glasgow series a spontaneous abortion rate of 1.4% was reduced to 0.2% by excluding those that occurred more than 2 weeks after the amniocentesis and that were not thought to be related to the procedure (McNay & Whitfield 1984).

The UK collaborative study (Medical Research Council 1978) showed an excess of abortion subsequent to amniocentesis of between 1.0-1.5%. However this study may have been biased by the inclusion of some controls at later gestational ages than their amniocentesis counterparts, thus reducing the likelihood of miscarriage in the control group (Milunsky 1979, Crandall 1980). Furthermore, the indication for amniocentesis in 110 patients was an elevated maternal serum alpha-fetoprotein, thereby selecting for a population already at increased risk for pregnancy loss (Ferguson-Smith et al 1979). In a randomised study of 4606 women without known risk of genetic disease, whose ages ranged from 25-34 years, Tabor et al (1986) found a 1% increase in the spontaneous abortion rate after amniocentesis (1.7%) when compared to controls (0.7%). Raised levels of maternal serum AFP before amniocentesis, transplacental sampling and withdrawal of discoloured fluid were associated with an increased risk of spontaneous abortion. An unsuspected hazard of amniocentesis reported by several studies (MRC 1978, NICHD 1976, Tabor et al 1986) was an increased frequency of unexplained respiratory difficulties and postural deformities in the infants. In summary, the results of these studies suggest that the risk of fetal loss following second trimester amniocentesis does not exceed 0.5%, and that complications such as fetal injury, amnionitis and perinatal morbidity are uncommon (Mennutti 1989).

Genetic amniocentesis has traditionally been performed at around 16 weeks gestation, in the belief that at this stage of pregnancy the procedure is technically easier and that the number of viable fetal cells prior to this time may be insufficient to allow successful culture. The routine use of ultrasound guidance for the procedure and the recent reports of successful cell cultures from amniotic fluid samples obtained at earlier stages in gestation are suggesting that amniocentesis need not necessarily be delayed until the second trimester of pregnancy (Johnson & Godmilow 1988, Hanson et al 1987). However, the risk of spontaneous abortion at this stage of pregnancy has not

yet been assessed in a controlled trial and the culture success rate when the procedure is performed between 8 and 11 weeks is only 68% compared with 100% at gestations of 12 weeks or more (Rooney et al 1989).

1.4.e.ii. Chorion villus sampling.

Chorionic villus sampling (CVS) offers several important advantages over amniocentesis as a method of prenatal diagnosis. It can be performed in the first trimester of pregnancy which provides the possibility for earlier termination of pregnancy, thereby reducing the psychological trauma (Spencer & Cox 1987) and maternal morbidity associated with second trimester abortion (Binkin 1986). CVS may be performed by either the transcervical or transabdominal route. However the transcervical route has been reported to carry a risk of spontaneous abortion some three to six times that of amniocentesis (Lilford 1985) and use of the transabdominal technique is increasing (Smidt-Jensen & Hahnenmann 1984, Brambati et al 1987, Nicolaides et al 1987, Maxwell et al 1986).

The overall fetal loss rate in 41,521 cases worldwide using all techniques has been reported as 3.54% (Jackson 1988). The loss rates are related to operator experience. In centres with experience of more than 100 tests the figure is 3.3% compared to 6.2% in centres with experience of less than 50 cases. Of all methods transabdominal sampling appears to have the lowest loss rates (Jackson 1988, Nicolaides et al 1987) and in a randomised trial comparing transcervical CVS, transabdominal CVS and amniocentesis, the respective loss rates were 6.4%, 3.3% and 0.7% (Smidt-Jensen & Philip 1987).

However, estimates of fetal loss can only be interpreted usefully in studies that employ ultrasound scanning for assessment of fetal viability prior to performance of the procedure, since approximately 10% of patients requesting CVS will be found to be carrying a non-viable pregnancy (Green et al 1988). The incidence of spontaneous abortion after an ultrasound scan has demonstrated a viable pregnancy in the first trimester has been shown to range from 1.3 - 3.4% (Christianens & Stoutenbeek 1984, Wilson et al 1985, Gilmore & McNay 1985, McFadyen 1985, Cashner et al 1987, Mackenzie et al 1988). This figure is higher with increased maternal age, earlier gestational age and previous spontaneous abortions. Below the age of 30 years, the spontaneous fetal loss rate is 1.5% rising to 4.5% between 35 and 39 years (Wilson et al 1985). In patients with a history of spontaneous abortion the loss rates are significantly higher (5.5%) compared with patients with no such

history (0.6%), and the spontaneous loss rate at less than 10 weeks gestation may be as much as three times that recorded after 10 weeks (Mackenzie et al 1988).

The preliminary results from the Canadian randomised trial (1989) comparing CVS to amniocentesis suggest that the potential difference in fetal loss rates is not more than 2.4% for women 35 years of age and over with a viable fetus at the time of the procedure. Although the fetal loss rates before 28 weeks gestation were not significantly different in CVS and amniocentesis patients (0.6%), there were more late losses after 28 weeks in the CVS group, for which at present there is no obvious explanation. By contrast, the results of a seven centre collaborative study in the USA (Rhoads et al 1989) reported that the excess of fetal loss in the patients who underwent CVS occurred before 16 weeks gestation (0.9%) and suggested that the loss rates after 16 weeks gestation were of equal frequency in the CVS and amniocentesis groups. The rate of loss of chromosomally normal fetuses after CVS was 10.8% among women in whom three or more attempts were made at sampling, compared to 2.9% in those in whom only one attempt was necessary. This study concluded that CVS is a safe and effective technique for early prenatal diagnosis of cytogenetic abnormalities, but that it probably entails a slightly higher risk of procedure failure and of fetal loss than does amniocentesis. The results of the UK randomised trial conducted by the MRC are still awaited.

1.4.f. Contraception.

1.4.f.i. Oral contraceptive usage.

The overall frequency of chromosomal abnormalities in abortuses amongst oral contraceptive pill (OCP) users has been shown to be marginally increased when compared to non-users (Boué et al 1975, Lauritsen 1975, Klinger 1976). An increase in triploidy was reported by Carr (1970), but later studies controlling for maternal age, social class and duration of contraceptive use could not attribute the increase to any specific abnormality (Alberman et al 1976). This finding led to suggestions that there may be an increase in early pregnancy losses among women conceiving shortly after stopping the pill, and that the temporary reduction in fertility of women coming off the pill (Vessey et al 1978) could be explained by unrecognised early miscarriages. Alternatively, the slight increase in chromosomal abnormalities may reflect a decrease in the risk of spontaneous abortion of chromosomally normal fetuses in OCP users (Alberman et al 1976).

Three large prospective studies have reported a slight decrease in the risk of spontaneous abortion in women using the OCP. In the Royal College of General Practitioners study (1976), spontaneous abortions were reported in 9.4% of former pill users and 10.4% of controls. There was a small increase in risk noted in women over the age of 35 years. In the study by Harlap et al (1980) former OCP users experienced lower than expected frequencies of loss at all stages of pregnancy, but a slight increase in first trimester abortions was noted after OCP failures, although no increase was evident when OCP usage continued until the last withdrawal bleed before conception. Vessey et al (1978) noted an 11.3% incidence of spontaneous abortion among former pill users and 12.2% in women using other forms of contraception or no contraception. A lower rate of multiple pregnancies among former users was observed, but among unplanned pregnancies, in which there was no interval between OCP use and conception, the rate of multiple pregnancies was significantly higher when compared to non-user controls.

In a retrospective study by Rothman (1977) no differences in the rate of abortion after short term and long term OCP usage was evident but a twofold increase in twinning rates for long term users who became pregnant soon after stopping the OCP was noted.

In summary, OCP usage does not appear to influence the risk of spontaneous abortion significantly nor its recurrence (Risch et al 1988). The widely quoted drug manufacturers advice that women should avoid conception for two to three months after stopping the OCP is not based on hard data.

1.4.f.ii. Intra uterine devices.

The efficacy of intra uterine contraceptive devices (IUCD) is ascribed to alterations in the uterine "milieu" making the conditions for implantation unfavourable (Tamaya et al 1976) and with modern techniques, raised luteal levels of hCG have not been demonstrated in women with IUCD's (Sharpe et al 1977, Keller and Soyka 1978). It has been suggested that the presence of an IUCD preferentially selects against the development of aneuploid conceptions (Honoré 1980). Severe growth disorganisation is less common in IUCD associated abortions and congenital abnormalities have not been reported in babies born with an IUCD in situ (Rosenfield 1978).

In general, the risk of spontaneous abortion is not increased after the removal of an IUCD (Vessey 1974, Foreman et al 1981) although in one study

(Harlap et al 1980a) a small increase in the risk during the first trimester of pregnancy was noted. Pregnancies conceived following IUCD failures are associated with a 50% risk of spontaneous abortion, predominantly in the second trimester of pregnancy, when the device remains in place (Tatum et al 1976, Vessey 1974, Harlap et al 1980a). Ascending infection can be demonstrated in 95% of cases (Eisinger 1976, Foreman et al 1981). but there is no significant association with one particular type of device (Harlap et al 1980a, Foreman et al 1981). The risk of abortion and infection is markedly reduced if the IUCD is removed or expelled early in pregnancy (Eisinger 1976, Foreman et al 1981, Tatum 1976) and these authors advocate removal of the device whenever the threads are still visible at the cervical os.

1.4.f.iii. Other methods of contraception.

In the comparative study of Harlap et al (1980a), the risk of spontaneous abortion in women using barrier methods was lower than average although there have been previous reports of an excess risk of abortion associated with spermicide creams (Oechsli 1976). There was no excess risk of miscarriages following rhythm failures.

1.4.g. Induced abortion.

The possibility that induced abortion increases the risk of spontaneous abortion in a subsequent pregnancy has received considerable attention since the introduction of legalised abortion to the United Kingdom in 1967, and the United States in 1973. Several studies conducted before the mid seventies demonstrated an increased incidence of second trimester spontaneous abortion and premature delivery in pregnancies which followed vaginal termination of pregnancy (Klinger 1970, Richardson & Dixon 1976). One study concluded that the high risk of cervical incompetence following forced dilatation of the cervix warranted fortnightly digital assessment of the cervix in subsequent pregnancies (Wright et al 1972).

More recent studies have been almost unanimous in their conclusions that legally conducted first trimester induced abortion carries no significantly increased risk of subsequent spontaneous abortion (Daling & Emmanuel 1977, Kline et al 1978, Harlap et al 1979, Obel 1980, Chung et al 1982). Intervention at an earlier gestation and improved techniques of abortion, such as the use of laminaria (Harlap et al 1979), have probably contributed to these improved results. In addition, the more recent studies have controlled for variables such

as maternal age, gravidity, social class, smoking and alcohol use, all of which factors bear a relationship to spontaneous and induced abortion.

Two independent studies have shown that young women who have had an induced abortion behave identically to primigravidae in their second pregnancy (Hogue 1982, Daling & Emmanuel 1977). Since young primigravidae are at greater risk of perinatal morbidity, particularly low birth weight, Daling (1977) suggested that induced abortion in women of less than 20 years of age had a less deleterious effect on subsequent reproductive outcome than the natural completion of a first pregnancy at this age.

Multiple induced abortions have been associated with an increased risk of ectopic pregnancy (Panayotou et al 1972), spontaneous abortion and premature delivery but in the most recent report by Kline et al (1986), no adverse short or long term sequelae of multiple induced abortions could be identified in women whose first induced abortion took place after 1975.

1.4.h. Endometriosis.

The relationship between endometriosis and spontaneous abortion has not been established. Retrospective studies indicate that the incidence of spontaneous abortion may be increased in the presence of untreated endometriosis (Petersohn 1970, Olive et al 1982). Most studies have centred upon series of women being treated for infertility (Wheeler et al 1983, Naples et al 1981) and report a marked decrease in the incidence of abortion after diagnosis and treatment, although no untreated control groups were included. The improved pregnancy outcome is most noticeable after conservative surgical treatment of endometriosis (Naples et al 1981) but it is not understood whether the significantly poorer pregnancy outcome after medical treatment can be attributed to the disease or the treatment. As pointed out by Huisjes (1984), endometriosis may be a neglected cause of abortion since the condition can be diagnosed in 15-20% of menstruating women (Chalmers 1975).

1.4.i. Miscellaneous.

Socio-economic background may affect the incidence of spontaneous abortion, the lower social groups having a higher abortion rate (Peterson 1968, Hemminki et al 1980b). Shapiro et al (1971) found a significantly higher abortion rate among non-whites compared to whites, but this racial association was not confirmed by the studies of Naylor and Warburton (1979). There appears to be no seasonal variation in the incidence of abortion after

controlling for the seasonal variation in the conception rate (Warren et al 1980), despite an earlier report to the contrary (McDonald 1971).

Multiple pregnancies are aborted more frequently than singletons (Livingstone & Poland 1980). Routine ultrasound in early pregnancy has demonstrated that the number of multiple pregnancies is higher than that which would be expected from the frequency at birth. The exact rate of early twin pregnancies has not been determined conclusively, since there is little prospective data documenting the incidence of "vanishing sacs". However, estimates of an abortion rate in multiple pregnancies of one in three have been suggested, not considering those continuing as singleton pregnancies (Huisjes 1984).

1.5. Recurrence risks.

1.5.a. The lack of data.

Although most authors agree that there is an increased risk of spontaneous abortion for a woman after a previous abortion, no generally accepted figures for the risks of recurrence are available. Spontaneous abortion is undoubtedly the commonest complication of pregnancy, since calculating from Warburton and Fraser's (1964) data, approximately 25% of all women who become pregnant will experience one or more clinically recognisable abortions. Hence, trying to establish the cause, and assessing the risk of a subsequent pregnancy loss for women presenting with a history of an early abortion is a recurrent problem for the clinician.

What advice should be given to a woman who has experienced pregnancy losses and is planning to embark upon further pregnancies?. The answer to this apparently simple question has proved elusive, undoubtedly reflecting the inconsistencies in terminology, incidence and aetiology already discussed. In day to day practice, it is usually assumed that the cause is a non-recurrent one and the affected woman is usually counselled that the chances of her next pregnancy being successful are higher than her risk of miscarrying again. Not surprisingly, these assumptions are rarely considered satisfactory by the woman who has aborted repeatedly and who is not prepared to accept that she has been statistically unlucky.

1.5.b. Previous studies of recurrence risk.

There are many studies in the literature which have attempted to put figures on the recurrence risk of an abortion after the first, second, third or subsequent pregnancy loss experienced by a woman. The results are at considerable variance because they are directly dependant on the population sample and the method of data analysis employed. Repeated pregnancy loss has been estimated as affecting up to 0.5% of all couples (Bishop 1937, Javert et al 1957, Stenchever 1980), but the definition of recurrent pregnancy loss is not always comparable. Some authors maintain that only couples with three or more spontaneous abortions should be included in the definition (Speert 1954, Huisjes 1984, Glass & Golbus 1978), whereas many other studies have included couples with 2 pregnancy losses (Tho et al 1979, Byrd et al 1977, Poland et al 1977, Harger et al 1983).

If it is assumed that the incidence of spontaneous abortion in any pregnancy is 15%, and that the risk of abortion is the same for all couples, the statistical risk of a woman experiencing 2 successive abortions should be 2% and the chance of 3 successive abortions should be 0.34% (Huisjes1984), which is not dissimilar from the empirical figure of 0.5% quoted earlier. Although this kind of calculation is subject to considerable error, it can be argued that a large number of abortions are fortuitous and that even 3 or more abortions in succession may be coincidental. However, the concept of "recurrent abortion" implies that in a proportion of cases there may be a systematic cause, and that there are some couples who are at increased risk for spontaneous abortion.

Evidence supporting this argument is derived from 2 sources. The first are data in which couples are classified by the number of pregnancy losses they have experienced and which demonstrate that the risk of a subsequent abortion increases with the number of previous abortions in a woman's reproductive history, or with characteristics of an index abortion such as karyotype, gestation or morphology. The second type of evidence comes from studies examining various characteristics of couples experiencing multiple spontaneous abortions and comparing them with a suitable control population. The presence of consistent differences suggests that recurrent abortion is a distinct clinical entity. Furthermore, this type of study may provide insights into the aetiology of recurrent abortion and is discussed further (section 1.6; p 114).

1.5.b.i. Theoretical studies.

The first available data on the risk of recurrent abortion were based on theoretical assumptions. Malpas (1938) reasoned that all cases of spontaneous

Table 1.6

Probability of spontaneous abortion with a given history

Type of study	Number of previous abortions			
	0	1	2	3+
<u>Theoretical</u>				
Malpas 1938	17	21.6	38	73.0
Eastman 1946	9.6	13.2	36.9	83.6
<u>Retrospective</u>				
Stevenson et al 1959		16.8	19.2	26.2
MacNaughton 1964		20.3	44.4	58.0
Warburton & Fraser 1979	12.3	23.7	26.2	32.2
Naylor & Warburton 1979	11.0	22.7	28.6	33.3
Leridon 1976	15.2	22.0	35.3	
Poland et al 1977		19.0	35.0	47.0
<u>Prospective</u>				
Speert 1954				19.0
Boué et al 1975		13.8		24.9
Lauritsen 1976		13.2		32.5
Harger et al 1983			16.6	30.0
Fitzsimmons et al 1983			31.3	45.7

abortion could be grouped into two categories, those resulting from non-recurrent or random causes and those due to recurrent factors. Women who aborted from a non-recurrent cause were at no greater risk of aborting

subsequent pregnancies, since the random factor was unlikely to be repeated in a succeeding pregnancy except by chance. On the other hand, among women "harbouring" a recurrent factor all subsequent pregnancies would be affected by this factor and would spontaneously abort.

Using data from a study by Whitehouse (1929), reporting an abortion frequency of 18%, Malpas calculated that the proportion of women experiencing 3 or more consecutive abortions accounted for less than 1% of this figure and that the remainder were due to random causes. Based on these assumptions he constructed a mathematical model to predict that the risk of abortion would be 22% after a single abortion, 38% after two abortions and 73% after three abortions.

Eastman (1946) modified these data by assuming that 10% of all pregnancies would end in spontaneous abortion, 9.6% from random causes and 0.4% from recurrent causes and calculated the risks of future abortion to be 13%, 37% and 84% respectively after one, two or three previous abortions. Both authors emphasised the significant difference in prognosis between a woman who has had two consecutive abortions and a woman who has had three consecutive losses, concluding that after three successive abortions "the presence of a recurrent factor may be inferred with reasonable certainty" and that the term "recurrent abortion" should be reserved for patients experiencing three or more consecutive abortions.

1.5.b.ii. Retrospective studies.

More optimistic figures for future pregnancy outcome were presented by Warburton and Fraser (1964) (Table 1.6) who interviewed 2134 women referred to a medical genetics unit in Montreal between 1952 and 1962, after the delivery of a live born child. They excluded the recruitment pregnancy from the obstetric histories of these women and analysed retrospectively 10,000 of their accumulated pregnancies. This study concluded that the risk of a further abortion after 1, 2 or 3 previous abortions was 23.7%, 26.2% and 32.2% respectively. However, these figures included all women who had experienced previous abortions, whether or not a successful pregnancy had intervened and since recruitment was dependant on the woman having achieved a viable pregnancy, "abortion-only" sequences were selected out.

Similar risk figures (Table 1.6) were later reported by Naylor and Warburton (1979) who examined 14,000 pregnancy histories obtained at antenatal registration in 13 hospitals participating in a collaborative perinatal

study between 1959 and 1966. Once more the recruitment pregnancy was excluded from the analysis, but since these pregnancies were registered at 20 weeks gestation or more, the vast majority of the study pregnancies ended in live births. However, this analysis took into consideration both the number of previous abortions and the sequence of abortions and live births. Although the overall risk of abortion after three or more abortions was recorded as 33.3%, amongst the 51 histories with three consecutive abortions the risk of a further abortion was 45%.

In contrast, the studies of MacNaughton (1964) and Poland et al (1977) recruited their patients following an abortion and only considered women with consecutive abortions, which most probably accounts for the higher recurrence risks reported in both studies (Table 1.6). In both of these studies the risk of a further abortion was higher for women who had never had a successful pregnancy (primary aborters) when compared to the risk experienced by women who had achieved a viable pregnancy in the past (secondary aborters).

These authors emphasised that studies estimating the recurrence risk of abortion should discern between these 2 categories of patient if confusion in the interpretation of the results were to be avoided. Furthermore, since the risk of a further spontaneous abortion rose considerably between the second and third consecutive pregnancy loss, they suggested that evaluation of the patient should begin after she has suffered a second abortion, since these women have already entered a high risk category and should be considered as "potential recurrent aborters".

There are many more retrospective studies that could be quoted, since this type of study design has the advantage of accessing large amounts of data quickly. The variable figures quoted in Table 1.6 (p 109) illustrate clearly that the method of patient selection affects the results significantly. Moreover, retrospective studies are susceptible to memory and reproductive compensation artifacts discussed earlier (section 1.2.a.iii; p 17) and by definition are most likely to under-represent the contribution of early spontaneous abortions which did not require medical intervention.

1.5.b.iii. Prospective studies.

The first prospective study of the risk of recurrent spontaneous abortion was reported by Speert in 1954, who considered that the high recurrence risks predicted by Malpas (1938) and (Eastman 1946) were unfounded. In Speert's

study, only patients with a history of three or more consecutive spontaneous abortions of a fetus weighing less than 1,000 grams, or gestational age of less than 28 weeks were included. Among 17,500 obstetric patients receiving antenatal care in New York between 1948 and 1952, 121 patients were identified who fulfilled his criteria for recurrent abortion, and of these 121, 81% carried their subsequent pregnancy to viability. Women with a history of 3 consecutive pregnancy losses had a better prognosis for a successful pregnancy (89%) than women who had had 4 or more pregnancy losses (71%). The abortion sequence began with the first pregnancy in 63% of the patients and was preceded by a successful pregnancy in 37% of the cases. The outcome of the studied pregnancy was different for the primary recurrent aborters (74% successful) and the secondary recurrent aborters (93% successful) and the improved prognosis for women who had achieved a viable pregnancy antedating her abortion sequence was present throughout all the gravidity groups.

Two large prospective studies have examined the relationship between risk of spontaneous abortion and the karyotype of the index abortion. The studies of Boué et al (1975) and Lauritsen (1976) both concluded that there was an increased risk of abortion after a previous abortion and that this risk increased with the number of previous abortions (Table 1.6; p 109). However, they found that the karyotype of the abortion affected the magnitude of the risk of recurrence for an individual patient. When a fetus with a normal karyotype was aborted, this was followed by another abortion in 23% (Boué et al 1975) and 26% (Lauritsen 1976), whereas the risk in the karyotypically abnormal groups were 16.5% and 13% respectively. These results contrasted with earlier retrospective series which reported no difference in the rate of spontaneous abortion among chromosomally normal and abnormal abortions (Carr 1967, Boué et al 1973). However, Alberman (1975) recognised that it was necessary to control for the fact that mothers with chromosomally normal abortions tend to be younger than mothers with chromosomally abnormal abortions. When this was done it did appear that abortions with normal karyotypes were associated with a higher recurrence risk.

More recent prospective studies of the recurrence risk of spontaneous abortion have been centred on series of women undergoing investigation for possible causes of their repeated pregnancy losses and have reported a variable prognosis for subsequent pregnancy outcome. In some studies 2 consecutive abortions have formed the criterion for inclusion, whereas in others 3

pregnancy losses have been used. Frequently the small numbers of patients studied prevent statistical analysis. Furthermore, some patients in whom a demonstrable cause for the pregnancy losses has been identified have undergone treatment and their subsequent pregnancy outcome has been compared to the expectant management offered to those patients in the unknown aetiology group.

The studies by Harger et al (1983) and Fitzsimmons et al (1983) have been included in Table 1.6, since the number of patients studied were comparable and they both provide estimates of the risk of recurrence after 2 and 3 consecutive spontaneous abortions. Despite these study design similarities, the probability of a subsequent pregnancy ending in a further abortion is markedly different, again emphasising the difficulties encountered by the clinician trying to counsel a woman about her future risk of recurrent abortion.

1.5.c. The prognostic importance of reliable data.

The disparities between studies attempting to assess the risk of recurrence of spontaneous abortion after 1, 2 or 3 previous episodes of abortion have important clinical implications. The advice that should be offered to an affected couple about their prognosis for future pregnancy outcome is not clear. Although all reports are consistent in concluding that there is an increased risk of abortion after a previous abortion, the risk for any individual may be higher or lower, depending on specific circumstances such as age, parity and biological variables such as chromosomal abnormality of the conceptus. Most importantly, the evaluation of treatment regimes is impossible without reference to agreed figures for the rate of spontaneous resolution and adequate control data.

Despite the shortcomings of existing studies, it is notable that many authors have found the likelihood of a successful pregnancy following three previous abortions to be in the region of 60%, which leads many clinicians to question whether the implementation of any treatment is justifiable. However, expectant management for the patient who has undergone repeated pregnancy losses is rarely acceptable, and the doctor is frequently pushed into investigating and implementing treatment in a patient for whom no cause for the repeated losses can be demonstrated, and it may be argued, has been statistically unlucky. This point has been illustrated repeatedly over the last couple of decades by the public demand that develops for any proposed "new"

treatment option, and which frequently prevents the completion of adequately controlled clinical trials.

1.6. Studies of the characteristics of recurrent aborters.

Most studies of the characteristics of women experiencing recurrent spontaneous abortions have been based on small selected patient groups derived from an individual clinician's practice. The study designed by Strobino et al (1986), based on a large unselected series of consecutive abortions collected in New York City, aimed to identify women at increased risk of recurrence. These investigators identified factors which distinguished women experiencing recurrent spontaneous abortions from women experiencing a single abortion and women who had no abortions.

The case group included all women of gravidity 3 or more seeking medical care for spontaneous abortion. Any woman reporting three or more spontaneous abortions and fewer live births than abortions was defined as a "repeater". Any woman of gravidity 3 or more reporting only one spontaneous abortion (the study pregnancy) and at least one previous live birth was defined as a "sporadic". The control group were women registered for antenatal care, matched for age and payment status (private or public) who delivered at 28 weeks or later. A further comparison group of "multiparae" were selected from the control group and defined as women with at least 3 livebirths and no spontaneous abortions.

Using a stepwise discriminant multivariate analysis, 5 characteristics were noted to be consistently associated with recurrent abortion. The characteristics were present in women with both primary and secondary recurrent abortion.

- 1) Loss of a chromosomally normal conception. The frequency of chromosomally normal abortions was greater among "repeaters" (83%) than among "sporadics" (67%). In this series, there were no repeaters who had conceptions with unbalanced chromosomal rearrangements inherited from one of the parents.
- 2) Increased length of gestation at abortion. The mean gestational age at abortion was greater for "repeaters" compared to "sporadics". Among "repeaters", 45% of fetal losses were at 14 weeks gestation or later, compared to 30% among the sporadics ($p < 0.01$). Because the proportion of chromosomally normal conceptions increases with gestational age, the excess of normal

conceptions among "repeaters" described above would be sufficient to raise the mean gestation at abortion. However, when only chromosomally normal abortions were considered, the mean gestation at abortion remained significantly higher in "repeaters".

3) Conception delay. A greater proportion of "repeaters" reported an interval to conception of more than one year prior to the study pregnancy

4) History of prematurity and low birthweight infants. In their previous reproductive history, "repeaters" had a greater proportion of previous preterm births (deliveries at less than 36 weeks gestation) and low birth weight infants (weighing less than 2,500 grams) than did "sporadics" or "multiparae". Furthermore, these low birthweight infants weighed significantly less than the low birthweight offspring of "sporadics" and "multiparae". Among term births (36 weeks or later) the frequency of low birthweight infants was again higher in the repeater group, suggesting that some of the excess low birthweight may be due to poor intrauterine growth as well as shortened gestation.

5) A diagnosis of cervical incompetence. Since this diagnosis is more likely to be sought and made in women with a reproductive history of repeated abortions and preterm births, the strong association noted in this study is difficult to interpret. Interestingly, when all the recurrent aborters who had had this diagnosis made were excluded from the analysis, the positive association between the other 4 factors and repeaters without a diagnosis of cervical incompetence remained.

The results from this study suggested that the reproductive characteristics of "repeaters" and "non-repeaters" differ significantly, lending support to the view that recurrent spontaneous abortion is a distinct clinical syndrome. Since three of these five characteristics (gestational age at abortion, delay in conceiving and previous preterm birth) are generally known at the time a woman presents with a first spontaneous abortion, the authors suggested that these 3 risk factors could be usefully used to predict future pregnancy outcome and identify an at-risk group of women before they embarked upon a second pregnancy. In cases where cytogenetic studies of the abortus are available, the finding of a normal karyotype increases the risk of subsequent abortion. However the diagnosis of cervical incompetence does not add to the evidence that recurrent abortion is a syndrome, since this diagnosis is confounded by a history of repeated pregnancy losses.

The study of Reginald et al (1987) also demonstrated that the prevalence of preterm delivery, perinatal death and small for gestational infants was significantly higher among babies born to women with a history of secondary recurrent miscarriage. These authors suggested that defective placentation in early pregnancy is one explanation for these associations and since pregnancy failures among secondary aborters are frequently partner specific, it is possible that the underlying mechanism may be immunologically mediated.

1.7. Studies of the aetiology of recurrent spontaneous abortion.

The relative importance of different aetiological factors in recurrent spontaneous abortions have been the subject of numerous studies. Determining the frequency of established and potential causes has been considered a useful method of obtaining information on the prognosis for subsequent pregnancies and the possible benefit of therapeutic regimens. A wide range of investigations and diagnostic tests have been employed, frequently dictated by the special interests of the investigators, and the results variably interpreted to implicate the relative importance of genetic, anatomical, infective, endocrine and immunological abnormalities as causal mechanisms for the recurrent abortions. Some studies have investigated women after two pregnancy losses (Harger et al 1983, Byrd et al 1977, Fitzsimmons et al 1983), or after one abortion associated with a phenotypically abnormal child (Tho et al 1979) while others have included women with three or more abortions only (Stray-Pedersen 1984). Whereas some authors have emphasised that the incidence of positive investigations are dependant on whether the patient is a primary or secondary recurrent aborter, or has experienced first or second trimester abortions, detailed characteristics of the population studied are unspecified in other reports.

The majority of studies have concluded that the incidence of detectable causes for the abortions increases with the number of previous abortions that an individual patient has had. However, even after extensive investigation a significant number of patients remain in whom no aetiology can be demonstrated. Table 1.7 illustrates the varied incidence of detectable aetiological factors in couples with recurrent abortion in the most frequently cited studies.

If "genetic aetiology" includes structural chromosomal and multifactorial abnormalities such as neural tube defects, the incidence may be as high as 25% (Tho et al 1979), whereas if the definition is limited to parental

balanced translocation carriers an incidence of 3% of individuals is noted (Stray-Pedersen 1984, Simpson 1981). The figure is higher if chromosomal variations such as pericentric inversions, satellites on acrocentric chromosomes and additional mosaic cell lines are included (Harger et al 1983), increasing to 14% in individuals with a history of abortion plus fetal malformation (Byrd et al 1977).

Table 1.7.

Studies of the incidence of aetiological factors in women with recurrent abortion.

Aetiological factor	Tho et al 1979 n=100	Harger et al 1983 n=155	Stray-Pedersens 1984 n=195	Parazzini et al 1988 n=158
Genetic	25%	8%	3%	5%
Anatomical	15%	27%	28%	30%
Infective	-	48%	15%	-
Endocrine	23%	2%	5%	5%
Other	-	8%	6%	-
Unknown	37%	32%	44%	60%

The diagnosis of an anatomical abnormality is usually dependant on an individuals interpretation of X-ray or hysteroscopic findings. The incidence is highest in studies which have included uterine fibroids and retroversion as causal factors (Stray-Pedersen 1984, Parazzini et al 1988). In the 2 studies in Table 1.7 in which infectious causes were sought, the incidence of positive endometrial and cervical cultures of *ureaplasma urealyticum* and *mycoplasma hominis* were high, but in neither study was a causal link definitively shown.

Similarly, when histological evidence of corpus luteum deficiency was sought the incidence of endocrine abnormalities was reported more frequently (Tho et al 1979) than when serum estimations of progesterone, glucose tolerance and thyroid function were investigated. The subgroup "other" includes a variety of investigations used by single studies ranging from anti-nuclear antibody testing (Harger et al 1983) to "sperm factors" and systemic disorders such as ulcerative colitis (Stray-Pedersen 1984).

With such non-uniform investigations it is not surprising that the "unknown aetiology" group has a variably reported incidence, since patients included in this category in one study may be classified alternatively in others.

1.8. Influence of aetiological factors on subsequent reproductive performance.

Several studies have suggested that determining the aetiology of a woman's recurrent pregnancy losses provides useful prognostic information for the outcome of subsequent pregnancies, since this method will identify some causes of repeated abortion for which appropriate treatment may improve the outcome. Some studies have treated certain categories of patients, whilst others have adopted an expectant management policy. There are many reports of empirical treatment with antibiotics or hormonal supplementation for patients in whom no identifiable cause can be found. Table 1.8 illustrates the subsequent successful pregnancy outcomes in three studies in which the patients were classified into those in whom the aetiology of their abortions had been established and those in whom it had not.

Table 1.8.

Comparison of pregnancy outcome in women with recurrent abortion of known and unknown aetiology.

	Tho et al 1979	Harger et al 1983	Stray-Pedersens 1984
Total no couples	100	155	195
Aetiology established	63	106	110
Live births/pregnancies (%)	38/63 (60%)	53/75 (71%)	56/70 (80%)
Unknown aetiology	37	49	85
(%)	(37%)	(32%)	(44%)
Live births/pregnancies (%)	23/37 (62%)	24/31 (77%)	40/61 (66%)

Within the known aetiology group, all three studies performed surgical correction for uterine and cervical structural abnormalities but the incidence of subsequent successful pregnancies varied from 60% - 80%. In the "endocrine" subgroup Stray Pedersen treated all patients with injections of

HCG and reported a 75% incidence of live births, whereas Tho used progesterone supplementation for this subgroup and reported a 91% live birth rate. Subsequent successful pregnancy outcomes of 70%-80% were reported in patients with positive infective screens treated with antibiotics by Harger and Stray-Pedersen. Interestingly, the greatest difference in subsequent pregnancy outcome (ranging from 32% to 100%) was noted amongst the genetic group for whom no treatment was available. However these large percentage differences within one subgroup of patients did not alter significantly the outcome of the known aetiology groups overall, again illustrating the inherent variations in the populations studied.

In the absence of suitable control groups, the pregnancy outcomes in the known and unknown aetiology groups have been compared. It is notable that all three studies cited in Table 1.8 demonstrate no significant differences in subsequent successful pregnancy outcome between women with established aetiologies for their pregnancy losses and women in whom the aetiology remains unknown. It is possible that the similarly good pregnancy outcome in the unknown aetiology group is due to the inclusion of some women who do not have a truly recurrent aetiology for their pregnancy losses, or in whom the cause was not identified but was influenced by empirical treatment. In the subsequent pregnancies reported by Tho et al (1979), 22 women in the unknown aetiology group received pre-pregnancy treatment with tetracycline to eliminate the possibility of mycoplasma infection and as soon as the pregnancy test was positive they were given progesterone suppositories. The other 15 patients in the unknown aetiology group received no treatment. The successful outcome in the treated group was 73% compared to 47% in the untreated group (Table 1.9).

An alternative explanation is that the prognosis for these patients may be improved by the medical attention they received whilst participating in the study. This argument receives strong support from the study of the Stray-Pedersen's who reported 61 subsequent pregnancies among the 85 couples with no detectable abnormalities for their recurrent losses. Thirty-seven of these pregnancies were selected to receive "tender loving care", consisting of psychological support, weekly hospital visits and bed rest for at least two weeks at the gestational period in which they had experienced their earlier abortions. The remaining 24 patients did not receive any specific ante-natal care. In the treated group 32 women (86%) carried their pregnancies to term but in the untreated group only eight women (33%) had successful

pregnancies. The difference in the two success rates was highly statistically significant ($p < 0.001$).

Table 1.9.

Recurrent abortion of unknown aetiology. Pregnancy outcome in treated and untreated women.

		No.of pregnancies	Successful pregnancies	%Successful
Tho et al 1979	Total	37	23	62
	Treated	22	16	73
	Untreated	15	7	47
Stray-Pedersen 1984	Total	61	40	66
	Treated	37	32	86*
	Untreated	24	8	33*

* $P < 0.001$

1.9. Definition of recurrent abortion.

In this account primary recurrent spontaneous abortion will be defined as the consecutive loss of three pregnancies before 20 weeks gestation and secondary recurrent spontaneous abortion as the consecutive loss of three pregnancies after the delivery of a live child.

The term "recurrent abortion" implies that recurrent episodes of early pregnancy loss have a systematic cause but this need not be so. However, a single persisting cause is likely to underly successive abortions in only a proportion of women since spontaneous abortion is common and the aetiology very varied (James 1961, Warburton & Fraser 1961). The distinction between a woman having recurrent abortions from a single persisting cause and a woman having repeated abortions from different causes is difficult. However, there do seem to be factors distinguishing some women with repeated abortion from others, suggesting that in addition to random causes, that there are an unknown proportion of cases in which specific components are involved in this type of reproductive failure and that "recurrent abortion" is a distinct clinical entity.

1.10. Purposes of this study.

The lack of prospective data on the incidence of sporadic and recurrent spontaneous abortion prevents the clinician from establishing the risk of recurrence and makes it impossible to assess the efficacy of treatment regimes.

There were four principal objectives to this study. The first aim was to determine, prospectively, the incidence of spontaneous abortion in a well documented population of women recruited prior to pregnancy. The study included nulligravidae, multigravidae and a group of recurrent aborters. Having established the incidence of abortion in this population, the study aimed to identify factors predisposing to pregnancy failure.

The results of the first part of the study demonstrated that past reproductive history is an important predictive factor for the outcome of a subsequent pregnancy. It is possible that some factor which allows pregnancy to continue is lacking in those women whose pregnancies are unsuccessful. The second aim of this study was to explore the possibility that anti-paternal cytotoxic antibody (APCA) was a marker for the "missing factor". This part of the study was designed to establish the incidence, time of development and significance of anti-paternal lymphocytotoxic antibodies in successful pregnancies and spontaneous abortions.

The third aim was to assess the efficacy of immunisation treatment with paternal white cell infusions in women with recurrent spontaneous abortion. The outcome of their subsequent pregnancies was compared with a control group of recurrent aborters who did not undergo treatment.

Observations made in the third part of the study suggested that women with a history of recurrent abortion and relative infertility have a particularly poor prognosis. The final part of this study examined the hypothesis that this was due to an endocrine imbalance and investigated whether the measurement of serum luteinising hormone concentrations before pregnancy was a useful prognostic test for the outcome of a subsequent pregnancy.

CHAPTER 2.

EARLY PREGNANCY LOSS FIELD STUDY.

Early Pregnancy Loss Field Study.

2.1. Introduction.

Spontaneous abortion is the commonest complication of pregnancy affecting about 25% of all women who become pregnant (Warburton & Fraser 1964) and assessing the risk of subsequent pregnancy loss is a recurrent problem for the clinician. In practice, it is usually assumed that the cause is non-recurrent and the patient is reassured that the chance of her next pregnancy continuing successfully is higher than the chance of her miscarrying again. Quantifying the risk to an individual patient of pregnancy losses after repeated abortions is even more difficult, since opinions differ widely as to whether patients who have experienced consecutive pregnancy losses suffer from a distinct clinical disorder or are simply unlucky statistically (Huisjes 1984, Malpas 1938). The lack of data also makes it impossible to assess the efficacy of treatment regimes.

One of the problems involved in establishing accurate figures is the bias introduced by patient selection. Most reports have been retrospective, hospital-based studies of pregnant women, because this sampling method provides a quick way of obtaining a large data pool. However, this approach provides no meaningful information about loss in first pregnancies (Naylor 1974, Naylor & Warburton 1979) In prospective studies, in which a declared pregnancy forms the basis for recruitment, early abortions that do not require medical intervention will be underrepresented (Roth 1963, Harlap et al 1980).

The Cambridge Early Pregnancy Loss (EPL) field study was undertaken with three aims: 1) to determine the incidence of spontaneous abortion in a well documented population recruited prior to pregnancy; 2) to identify factors predisposing to spontaneous abortion; and 3) to assess the risk of recurrent abortion. The study population afforded an ideal opportunity for accurate collection of this data, since all patients in this catchment area receive their antenatal care and undergo delivery at a single hospital.

2.2. Patients and Methods.

Women in the Cambridge hospital catchment area who were planning to become pregnant were enrolled into the study by a local radio and poster appeal. Patients were also drawn from postnatal and infertility clinics at Addenbrookes hospital together with patients who had miscarried during the

study period at the hospital. Further patients who had been referred specifically for pre-pregnancy counselling were included in the study.

All volunteers were interviewed before they became pregnant, in order to document their general medical, social, gynaecological and obstetric history and to explain the nature of the study. They were asked to report immediately following the first missed menstrual period. As soon as a pregnancy was suspected, the patient was seen and underwent an ultrasound examination using a real time abdominal sector scanner with a 2.5mHz transducer (Elscent ESI-1000, Israel). Early pregnancy blood samples were obtained from the patients and the serum tested for human chorionic gonadotrophin (Tandem ICON HCG, Hybritech, Europe S.A.). Patients developing symptoms of threatened abortion were seen immediately and further ultrasound examinations carried out.

For those patients whose pregnancy ended in spontaneous abortion, clinical and ultrasound parameters were recorded, to permit classification of the type of spontaneous abortion into one of the following categories: 1) Complete abortion - in which the uterine cavity was seen to be empty, as documented by the return of a midline linear endometrial echo. 2) Incomplete abortion - the appearance of thick irregular echoes in the uterine cavity. This diagnosis was confirmed retrospectively by histological examination of the products of conception after evacuation of the uterine contents. 3) Anembryonic pregnancy - when an empty gestational sac of volume greater than 3.0ml was present at 7 weeks gestation or greater. 4) Missed abortion - when fetal parts were identified but fetal heart activity had never been demonstrated. 5) Embryonic/fetal death - defined as a pregnancy in which fetal heart activity had been documented and which disappeared subsequently. Cytogenetic analysis of the products of conception was performed whenever tissue was available.

Serial ultrasound assessment of the pregnancy was continued at fortnightly intervals until 12 weeks gestation, after which arrangements were made to follow all continuing pregnancies throughout their antenatal course. Detailed records of any complications arising in those patients with ongoing pregnancies were completed at the time of delivery.

2.3. Statistical Analysis.

Comparisons between groups were made using 2×2 contingency tables. Fisher's exact test was applied when cells contained less than 5 samples. Odds

ratio and 95% confidence limits are given where appropriate (Gardner & Altman 1986).

2.4. Results.

2.4.a. Recruits.

Between February 1986 and July 1988, 132 nulligravidae and 498 multigravidae were recruited to the Cambridge Early Pregnancy Loss Study. By the 1st December 1988, 412 (66%) of the original 630 recruits had completed a pregnancy. Of these 412, 186 (45%) had responded to the radio and poster appeal, 120 (29%) had presented following a miscarriage at the hospital, 69 (17%) had been recruited from the infertility clinic, including 34 patients participating in the In Vitro Fertilisation (IVF) or Gamete Intra Fallopian Transfer (GIFT) programme. The remaining 37 women (9%) had been referred because of problems with one or more previous pregnancies or because of general medical disorders. Nineteen of these (5% of the total pregnancies) were classified as recurrent aborters, defined as having suffered 3 consecutive pregnancy losses before 20 weeks gestation. Of the 186 patients recruited by the radio appeal, 52 (28%) were nulligravidae, 72 (39%) had a history of successful pregnancies alone and only 17 (9%) had an obstetric history which contained a spontaneous abortion (4% of the total population).

During the recruitment programme, volunteers were contacted by telephone at six monthly intervals after enrolment if they had not already reported a pregnancy. Only 5 pregnancies were identified by this method. Two of these were to patients who had moved out of the area. Nine patients failed to conceive after unprotected sexual intercourse over a period of 18 months. A further 2 patients have been lost to follow-up. Attempts at pregnancy have been discontinued by 2 patients on the grounds of advanced maternal age. Hence, the careful follow up arrangements for all patients recruited to the study, resulted in a minimal number of dropouts (18) of which 50% were failures to conceive.

2.4.b. Clinical Outcome of 412 pregnancies.

There were 357 successful pregnancies, 50 spontaneous abortions, 2 ectopic gestations and 3 pregnancies requiring therapeutic termination for fetal abnormality. Spontaneous abortion was defined as the expulsion of a fetus before 20 weeks gestation or weighing less than 500 grams, in accordance

with the World Health Organization's recommended terminology (1977). A further three pregnancies were lost at 24, 26 and 27 weeks respectively and have been excluded from the subsequent analysis. Two of these were twin pregnancies, one complicated by placental abruption, the second by twin transfusion syndrome. The third pregnancy, in a woman with systemic lupus erythematosus, developed fulminating pre-eclampsia and placental infarction. The ectopic pregnancies and the therapeutic terminations have been excluded from the analysis of factors affecting the incidence of spontaneous abortion. Thus the subsequent analyses are based on 407 pregnancies, 50 spontaneous abortions and 357 live births.

Ultrasound examination allowed confirmation of pregnancy in 95% of the patients before the completion of the 8th gestational week, by which time 48% (24) of the abortions had occurred. The gestational ages at which the spontaneous abortions occurred are shown in Figure 2.1.

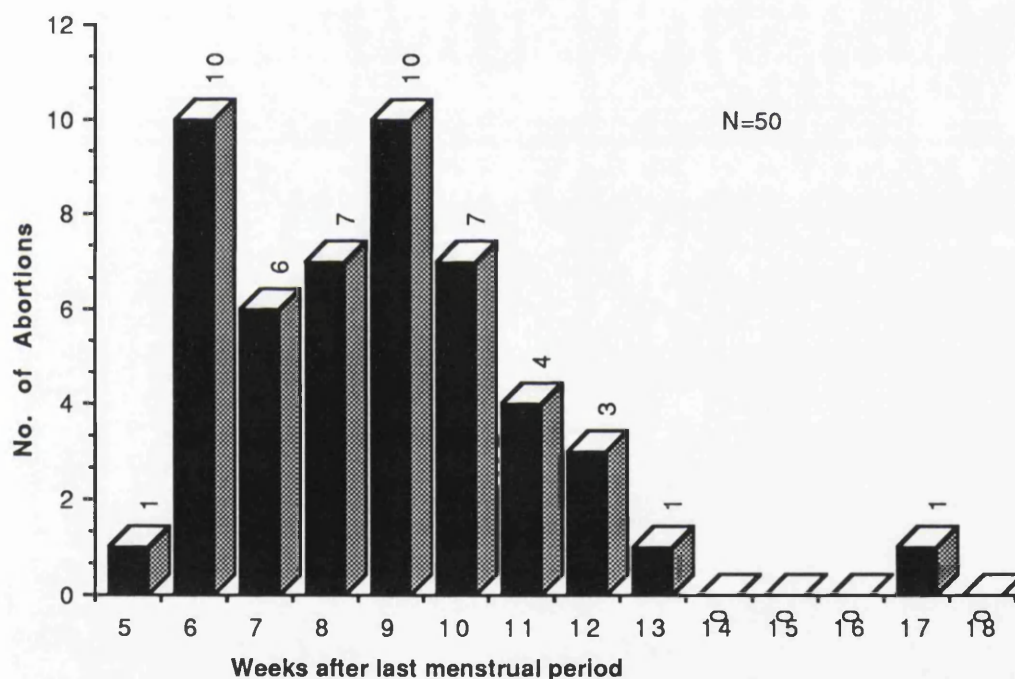


Figure 2.1 Gestational ages of 50 abortions

All but two of the 50 spontaneous abortions were declared in the first trimester of pregnancy, 14 complete, 4 incomplete, 11 anembryonic, 10 missed abortion and 9 embryonic/fetal death. The remaining 2 spontaneous abortions occurred at 13 and 17 weeks respectively. Both were classified as embryonic/fetal death.

Fetal karyotyping was performed on 24 of the abortuses (48%). There were 4 chromosomal abnormalities: one triploidy, one trisomy, one double mosaic and one unbalanced translocation. In 2 of the anembryonic pregnancies, culture failed because of maternal decidual overgrowth. Infected culture tubes prevented analysis of a further 2 abortuses, both missed abortions. All 11 embryonic/fetal deaths were analysed and had normal chromosome complements. These 11 pregnancy losses were all suffered by women who had experienced 1 or more previous abortions and whose last pregnancy had aborted.

2.4.c. Incidence of spontaneous abortion by gravidity.

The outcome of the 407 pregnancies grouped by gravidity is shown in Table 2.1. The overall incidence of spontaneous abortion for this population was 12%. Amongst the 87 primigravid pregnancies only 4 aborted (5%), whilst 46 of the 320 multigravidae suffered a spontaneous abortion (14%).

Table 2.1.
Outcome of 407 pregnancies grouped by gravidity.

Pregnancy outcome	Pregnancy Number					Total
	1st	2nd	3rd	4th	>5th	
Live	83	113	80	45	36	357
Abortion	4	16	14	9	7	50
Total No.	87	129	94	54	43	407
Patients						
Incidence of	5% ^a	12%	15%	17%	16%	12%
Abortion %						
					14% ^b	

The primigravid abortion incidence (a) is significantly different from the mean incidence in the multigravidae (b) $\chi^2=5.19$; $p=0.02$.

Analysis of pregnancy outcome by gravidity suggests that women embarking on their first pregnancy have the lowest risk of pregnancy loss ($\chi^2=$

5.19; $p=0.02$; OR=3.48; 95% CL: 1.2-10.2), and that gravidity is a useful measure of risk. However, when the multigravidae recruited following a recent miscarriage at the hospital were excluded from the gravidity analysis, (which takes no account of whether the previous pregnancies have been successful or unsuccessful) the increased incidence of abortion within the higher gravidity groupings was no longer statistically significant ($\chi^2=1.39$; $p=0.2$; OR=2.2; 95% CL: 0.7-8.1).

2.4.d. Non-influential factors.

The mean maternal age for the study population was 29.6 years (SD 4.5). There was no appreciable increase in the incidence of spontaneous abortion at either end of the reproductive age range, although only 3 patients were less than 20 years of age and only 25 were older than 38 years. The mean age of the 50 women who aborted was the same as the mean age of the live outcome group (29.7 ± 5.1 vs 29.6 ± 4.2 years). No significant association between the risk of spontaneous abortion and social class, smoking habits, drug ingestion, contraceptive usage, previous therapeutic termination of pregnancy or past medical history was identified.

2.4.e. Influence of infertility therapy.

In addition to the 69 patients recruited directly from the infertility clinic, a further 12 patients had received therapy for subfertility from their General Practitioners. In total, 81 pregnancies (33 primigravidae and 48 multigravidae) resulted from infertility treatment as follows: IVF - 21, GIFT - 13, ovulation induction therapy with clomiphene citrate - 30, LHRH therapy - 4, bromocriptine - 3, artificial insemination with either husband's or donor's sperm - 10. Amongst these 81 infertility patients there were only 13 spontaneous abortions (16%), which is not different statistically from the 11% loss rate recorded for the spontaneous pregnancy group (37 abortions within 326 conceptions; $\chi^2 = 0.93$; $p=0.3$). Only one of the IVF/GIFT pregnancies aborted, whilst 9 of the clomiphene induced conceptions (30%) ended in spontaneous abortion. One abortion followed bromocriptine therapy, one followed LHRH therapy and the remaining abortion was to a patient with male factor infertility.

However, only one spontaneous abortion (3%) occurred amongst the 33 primigravidae treated successfully for infertility, an incidence which is substantially lower than the 25% pregnancy loss noted for the multigravidae

treated for infertility (12 abortions in 48 conceptions; $\chi^2=5.47$; $p=0.02$; OR=11.1; 95% CL: 1.29-76.92).

2.4.f. Influence of past reproductive history on the risk of spontaneous abortion.

In Table 2.2, the 50 study pregnancies ending in a spontaneous abortion have been categorised by the patient's previous reproductive history and also subdivided into spontaneous conceptions (n=37) and those following treatment for subfertility (n=17).

Table 2.2.

Outcome of study pregnancy categorised by mother's reproductive history.

History	Number (%) of patients aborting		
	Entire group (n =50)	Spontaneous conceptions (n =37)	Assisted conceptions (n=13)
Last pregnancy aborted	40 (80%) ^a	31(84%) ^h	9(69%) ^l
Only abortions in the past	24 (48%) ^c	17(46%) ^k	7(54%) ⁿ
Only pregnancy aborted	12 (24%) ^e	7(19%)	4(31%)
Last pregnancy successful	5 (10%) ^b	3(8%) ⁱ	2(15%)
All pregnancies successful	3 (6%) ^d	2(5%)	1(8%)
Only pregnancy successful	3 (6%) ^f	2(5%)	1(8%)
Previous termination of pregnancy	2 (4%)	1(3%)	1(8%)
Primigravidae	4 (8%) ^g	3(8%) ^j	1(8%) ^m

a vs b: $\chi^2=46.7$; $p<10^{-6}$: OR=36; 95%CL=10.1-138.5

c vs d: $\chi^2=20.29$; $p=0.000007$: OR=14.5; 95%CL=3.6-46.2

e vs f: $\chi^2=5.02$; $p=0.03$: OR=4.9; 95%CL=1.2-20.0

a vs g: $\chi^2=49.72$; $p<10^{-6}$: OR=46; 95%CL=11.9-196.9

c vs g: $\chi^2=17.9$; $p=0.00002$: OR=10.6; 95%CL=3.0-40.9

h.vs i: $\chi^2=39.67$; $p=<10^{-6}$: OR=58.6; 95%CL:11.6-207.8

h.vs j: $\chi^2=39.67$; $p=<10^{-6}$: OR=58.6; 95%CL:11.6-207.8

j vs k: $\chi^2=11.58$; $p=0.00067$: OR=9.6: 95%CL:2.2-35.5

l vs m: $\chi^2=7.96$; $p=0.0047$: OR=2.1: 95%CL:2.1-266.4

m vs n: $\chi^2=4.51$; $p=0.045$: OR=14:95%CL:1.2-159.8

Of the 50 spontaneous abortions, 40 (80%) were suffered by women whose previous pregnancy had ended in an abortion. Twenty four women (48%) had only had unsuccessful pregnancies and 12 (24%) patients' sole previous pregnancy had ended in spontaneous abortion. In contrast, only 5 patients whose last pregnancy had resulted in a live birth aborted (10%). For the primigravidae, the incidence of spontaneous abortion was 8% (4/50), and for those women who had only had successful pregnancies in the past, the incidence was 6% (3/50). There were 2 spontaneous abortions among the 32 patients whose previous reproductive history contained a termination of pregnancy (6%). The 5 patients whose obstetric history included an ectopic gestation were excluded from this part of the analysis.

Although the numbers of patients in the subfertility subgroup are small, Fisher's exact test demonstrates no statistical difference between the 2 subgroups who conceived spontaneously and that following subfertility treatment, within the categories of reproductive history. However in both subgroups, the difference in the incidence of abortion between primigravidae and women who had suffered previous abortions is substantial (Table 2.2).

Since past reproductive performance appeared to be a significant contributory factor for the risk of abortion, the same analysis was applied to the study group as a whole. The risk of abortion classified solely on the patient's reproductive history for all 407 study pregnancies is shown in Table 2.3. The risk of abortion for the 326 pregnancies conceived spontaneously is shown separately in Table 2.4.

Patients whose last pregnancy had ended in an abortion had a 19% chance of aborting the study pregnancy, a significantly higher risk than primigravidae (5%), women whose pregnancies had all been successful (4%) and patients whose last pregnancy was live (5%). The risk for spontaneously conceived pregnancies only are similar although the confidence intervals are larger due to the reduction in sample size.

Table 2.3.

Effect of reproductive history upon the risk of spontaneous abortion - entire group (n=407).

History	No of patients aborting	Total number of patients	% Risk of abortion in study pregnancy
Last pregnancy aborted	40	214	19 ^a
Only abortions in the past	24	98	24 ^c
Only pregnancy aborted	12	59	20 ^e
Last pregnancy successful	5	95	5 ^b
All pregnancies successful	3	73	4 ^d
Only pregnancy successful	3	62	5 ^f
Previous termination of pregnancy	2	32	6
Primigravidae	4	87	5 ^g

a vs b: $\chi^2 = 8.32$; $p = 0.004$; OR= 4.1; 95%CL=1.5-12.2

c vs d: $\chi^2 = 11.2$; $p = 0.0008$; OR= 7.4; 95%CL=2.0-23.7

e vs f: $\chi^2 = 5.34$; $p = 0.02$; OR= 5.0; 95%CL=1.2-19.7

a vs g: $\chi^2 = 8.75$; $p = 0.003$; OR=4.8; 95%CL=1.6-13.4

c vs g: $\chi^2 = 12.69$; $p = 0.00036$; OR =6.7; 95%CL=2.1-24.1

e vs g: $\chi^2 = 7.39$; $p = 0.0065$; OR=5.3; 95%CL=1.5-20.8

Table 2.4.

Effect of reproductive history upon the risk of spontaneous abortion -
spontaneous conceptions only (n=326).

History	No.patients aborting	Total number of patients	% Risk of abortion in study pregnancy
Last pregnancy aborted	31	187	17 ^a
Only abortions in the past	17	78	22 ^c
Only pregnancy aborted	8	47	17 ^e
Last pregnancy successful	3	77	4 ^b
All pregnancies successful	2	58	3 ^d
Only pregnancy successful	2	49	4 ^f
Previous termination of pregnancy	1	29	3
Primigravidae	3	54	6 ^g

a vs b: $\chi^2 = 6.73$; p= 0.009; OR= 4.9; 95%CL=1.4-16.3

c vs d: $\chi^2 = 7.85$; p=0.005; OR= 7.8; 95%CL=1.6-32.8

e vs f: $\chi^2 = 3.03$; p=0.08; OR= 4.8; 95%CL=0.8-28.3

(Fishers exact test P=0.04)

a vs g: $\chi^2 = 3.34$; p=0.067; OR=3.4; 95%CL=0.9-12.5

c vs g: $\chi^2 = 5.34$; p=0.02; OR=4.7; 95%CL=1.2-17.7

In Figure 2.2, the abortion rate for women in the various reproductive categories is illustrated in a histogram.

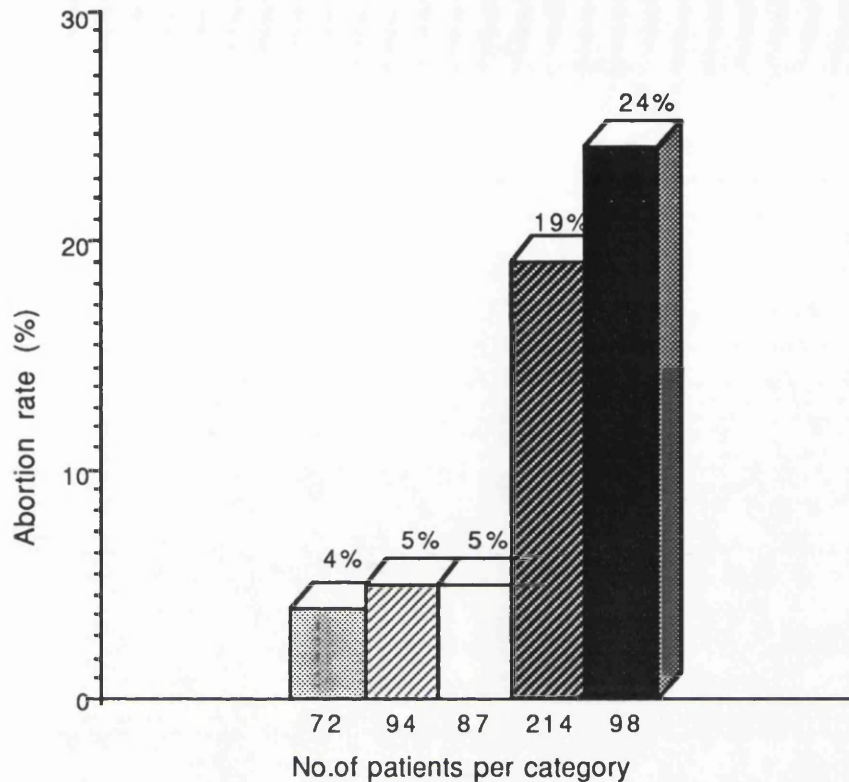







Figure 2.2. Histogram showing the abortion rate in women with a history of only successful pregnancies , whose last pregnancy was successful , primigravidae , women whose last pregnancy aborted , and women all of whose pregnancies aborted .

The greatest risk of abortion (24%), was for those patients whose obstetric histories contained abortions only. This increased risk was not due to the inclusion of potential recurrent aborters in the patient sample, since the risk of abortion was as high when the analysis of pregnancy outcome was confined to women of gravidity three or less ("All pregnancies successful" 3/67 (4%) compared with "Only abortions in the past" 21/91 (23%); $\chi^2 = 8.97$; $p=0.003$; OR=6.4; 95% CL: 1.7-21.6). Similarly, within this lower gravidity subgroup the high risk of abortion was maintained when the patients were categorised by the outcome of the last pregnancy (Last pregnancy successful 5/87 (6%) compared to last pregnancy aborted 25/126 (20%), $\chi^2 = 7.32$, $p=0.007$; OR=4.1; CL: 1.4-12.7). Furthermore, reanalysis of the data after excluding those patients who had been recruited following a recent miscarriage at the hospital

(n=120) demonstrated that the proportion of patients in the various subgroups detailed in Tables 2.2, 2.3 and 2.4 were unchanged.

The risk of abortion for a woman who has only ever aborted becomes cumulatively greater as the number of abortions increases from one (20%, 12/59), to two (28%, 9/32) and reaches 43% after three consecutive pregnancy losses (3/7), a trend which is also evident in those women who had had successful pregnancies in addition to their spontaneous abortions (Table 2.5).

Table 2.5.

Effect of previous successful pregnancies on the outcome of pregnancy

Obstetric History		% Risk of abortion	No. patients aborting/ Total No. patients
Only Abortions	1A	20	(12/59)
	2A	28	(9/32)
	3A	43	(3/7)
1 Successful pregnancy and Abortions	1A +1S	13	(5/40)
	2A+1S	19	(5/26)
	3A+1S	33	(4/12)
2 Successful pregnancies and Abortions	1A+2S	5	(1/20)
	2A+2S	13	(1/8)
	3A+2S	20	(1/5)

A=Abortion; S=Successful pregnancy

The presence of a live child in the past obstetric history appears to reduce the risk of abortion for that woman, but this influence is only significant statistically in those woman who have had 2 or more successful pregnancies in addition to their spontaneous abortions (1/20 vs 24/98; Fisher's exact test p=0.04)

Finally, the risk of abortion in any subsequent pregnancy, for a woman whose first pregnancy had a successful outcome (9%), is significantly lower than if her first pregnancy ended in an abortion (22%) irrespective of whether any intervening pregnancy ended in success or failure. (First pregnancy successful 14/150 compared to first pregnancy aborted 29/133; $\chi^2 = 7.57$; p=0.006; OR=2.7; 95% CL: 1.3-5.7).

2.5. Discussion.

It is generally quoted that 15% of clinically recognised pregnancies abort spontaneously (Warburton & Fraser 1964, Petersson 1968, Poland et al 1977). However, this figure may be an underestimate since the majority of studies have been based on data collected retrospectively from hospital populations (Javert 1957, Roth 1963, Harlap et al 1980). In these studies, early spontaneous abortions are likely to be underrepresented because symptoms of early abortion tend to be lighter and the patient is less likely to seek specialist advice (Warburton & Fraser 1964, Harlap et al 1980). On the other hand, prospective studies that recruit patients who are already pregnant may exaggerate the incidence of loss, since symptoms of threatened abortion will encourage earlier presentation of "at risk" pregnancies to the study (French & Bierman 1963, Shapiro et al 1971)

The overall incidence of early pregnancy loss of 12% in this study is slightly lower than that reported generally but agrees with a recent study where patients were also enrolled prospectively prior to pregnancy (12%) (Wilcox et al 1988). The study design suggests that this figure is an accurate reflection of the frequency of clinical spontaneous abortion in our study population, since it ensured that early abortions were reported. A diagnosis of pregnancy was achieved in 95% of the pregnancies before 8 weeks gestation, by which time 50% of the abortions had occurred. Furthermore, the drop-out rate of recruits was very low (18) of whom 50% were "failures to conceive". No attempt was made to assess the contribution of subclinical abortion. (Wilcox et al 1988, Walker et al 1988)

This demonstration from a prospective study, that a woman's risk of spontaneous abortion may be quantified by examining her past obstetric history has not been reported previously, and the table of risks (table 2.3; p 131) will be of use to the practising clinician. Primigravidae, women whose pregnancies have all been successful, and women whose last pregnancy was completed successfully, all have a low incidence of spontaneous abortion. The outcome of the previous pregnancy also is an important predictive factor for spontaneous abortion, for the risk of spontaneous abortion in women whose last pregnancy was unsuccessful was substantially higher than in women whose last pregnancy outcome was successful. In this regard, the use of gravidity grouping to classify pregnancy outcome can be misleading, since this method takes no account of whether the previous pregnancies had been

successful or unsuccessful, and encourages the clustering of women suffering several miscarriages within the higher gravidity groups which by definition will occur at progressively older ages.

Analysis of the data from women who had only ever aborted confirmed previous reports of a progressive increase in abortion risk after 1, 2 or 3 previous episodes (Naylor & Warburton 1979, Poland et al 1977, Leridon 1976, MacNaughton 1964). However the presence of a live child in the past obstetric history does reduce significantly the risk of abortion for that woman. This might explain why previous studies of recurrence risk, which have not taken previous pregnancy outcome into account, have quoted such variable figures (Warburton & Fraser 1964, Poland et al 1977).

The finding from this study that primigravidae have a low incidence of spontaneous clinical abortion (5%), has not been reported previously from a prospectively recruited population. Those studies which have concluded that abortion is more common in first pregnancies (Poland et al 1977, Awan 1974, Glass & Golbus 1978) are retrospective and suffer from the inadequacies referred to earlier. More recent attempts to establish the incidence of abortion in primigravidae have been made solely from assisted fertility programmes where the incidence of spontaneous abortion ranges from 12% to 45% (Chartier et al 1979, Lopata 1980, Edwards & Steptoe 1983). This study included 87 primigravidae of whom only 33 had received treatment for subfertility. It is interesting to note that in this study the incidence of abortion in primigravid spontaneous conceptions was 6% (3/54) whereas the corresponding figure for first pregnancies achieved after subfertility treatment was only 3% (1/33).

The suggestion that the method used to assist conception in subfertile couples might affect the frequency of subsequent pregnancy loss (Jansen 1982a) is confirmed in part by the results of this study, where the incidence of abortion following clomiphene therapy is more than twice the average. The possibility that the higher incidence of spontaneous abortion quoted for first pregnancies following infertility treatment reflects the more detailed periconceptual observation usually employed for such patients (Frydman et al 1981, Ben-Rafael et al 1988) does not apply to this study, since all our patients received the same early pregnancy surveillance. The finding that primigravid pregnancies, whether natural or assisted conceptions, share the same low risk of spontaneous abortion when compared to multigravidae, suggests that it is the past reproductive history, rather than infertility treatment per se, that influences the subsequent pregnancy outcome.

The characteristic J-shaped curve of fetal loss associated with rising maternal age (Roman 1980) was not evident in our study population, probably because there were insufficient patient numbers in the older age groups. The quoted increase in spontaneous abortion after age 35 is thought to reflect the increased risk of fetal chromosomal abnormalities, which is believed to account for 50-60% of spontaneous abortion (Boue et al 1975, Hassold et al 1980). In this study abortus material was obtained from 24 of the 50 cases of spontaneous abortion. Culture failure prevented analysis in 4 cases. Only 4 of the 20 abortuses karyotyped successfully were abnormal cytogenetically (13%). If it is assumed that a similar percentage of the fetal products not available for analysis were also abnormal (Hassold et al 1980, Boué et al 1975), the resultant figure of 26% from this study is substantially lower than would be expected, although this finding may reflect simply the small number of participants in this study over the age of 35 years (n=35). However, it was noted that all 11 of the abortuses which had been shown to have fetal heart activity prior to the abortion had normal chromosomal complements, and were carried by women whose previous pregnancy had aborted. Since women who experience recurrent miscarriages are more likely to have a chromosomally normal abortus (Strobino et al 1986) and the risk of an abortion following the loss of a fetus with a normal karyotype is higher than the risk incurred by women who have had karyotypically abnormal abortuses (Lauritsen 1976) the combined use of detailed ultrasound and cytogenetic analysis may provide a way of identifying women destined to become recurrent aborters.

The mechanism by which past obstetric history might influence the outcome of subsequent pregnancies is not clear. However it is important to note that the risk of aborting the study pregnancy for patients whose last pregnancy had a successful outcome is similar to that noted both for women whose pregnancies have only ever been successful and for the primigravidae. Thus, it might be postulated that a successful pregnancy does not exert a "protective effect", rather those patients who abort spontaneously do so because some factor which would allow pregnancy to continue is lacking. Persistent lack of this factor would lead to recurrent spontaneous abortion.

A number of hypotheses have been advanced in support of such an "absent-factor" type of mechanism. Blocking antibodies which can inhibit the mixed lymphocyte reaction (MLR), and are present in sera from multiparous women, are claimed to be either absent or present only in low titres in sera from women with recurrent abortions (Rocklin et al 1976, Stimson et al 1979).

Mowbray et al (1987) have suggested that a circulating anti-paternal cytotoxic antibody (APCA), whether naturally occurring, or induced by immunisation of the woman with paternal lymphocytes, is associated with successful pregnancy, and that the absence of the antibody is associated with an increased chance of repeated pregnancy loss. (See Chapter 3). It has also been suggested that increased sharing of HLA antigens (Komlos et al 1977, Gerencer et al 1979) or HLA linked antigens (Power et al 1983) between partners may be important in the aetiology of spontaneous abortion. It would be plausible to implicate this type of mechanism in pregnancy loss following previous spontaneous abortion, as women who share some HLA types with their partner would have a variable outcome of their pregnancy depending on the segregation of their genotype, but overall would be expected to do worse than couples with wholly dissimilar tissue types. This type of mechanism might also explain the observation from this study that a reproductive history containing only abortions produces a high risk of another abortion, but that this risk is reduced significantly when previous successful pregnancies have occurred in addition to the pregnancy failures. However McLaren's examination of controlled data from animal studies casts doubt on the original hypothesis (Clarke & Kirby 1974) on which these immunological theories are based, and subsequent clinical studies have also failed to substantiate the theory (Caudle et al 1983, Oksenberg et al 1984, Adinolfi 1986, Jazwinska et al 1987, Christiansen et al 1989).

Clarke et al (1975) have proposed that residual material in the uterus following a spontaneous abortion might affect a subsequent pregnancy. In their studies (Clarke et al 1975, Gardiner et al 1978) they postulated that trophoblastic cell "rests" influenced the outcome of subsequent pregnancies, since spina bifida and anencephaly were found to be more common in patients whose last pregnancy aborted when compared with matched controls in whom the outcome had been a normal baby. Furthermore, the interpregnancy gap was shorter in those women who had an abnormal baby compared with the matched controls. It is difficult to understand exactly how this mechanism would operate, since chorionic villi are unlikely to persist for longer than 3 weeks after an abortion (Stevenson & McClarin 1957) but it is perhaps noteworthy that the mean interval between spontaneous conceptions in our study population was 13.8 months for the 274 multigravidae who had successful outcomes compared with 7.6 months for the 46 multigravidae who aborted the study pregnancy.

The possibility that the results of this study reflect selection bias in the population studied demands careful evaluation. Although 45% of the patients were recruited from the radio appeal, and thus might bias the study toward recruitment of a self selected group of patients with a previous history of abortion, this was not found to be the case. Only 9% of this group had a history containing an abortion, whereas 28% were primigravidae and 39% had only had successful pregnancies in the past. The inclusion of patients following a recent miscarriage at the hospital (n=120) or who had experienced previous pregnancy problems (n=37), also could bias the results. However, after excluding these patients, the proportions in the various groups shown in tables 2.3 (p 131) and 2.4 (132) were unchanged. This hospital catchment area includes a group of middle class, well motivated patients who may well have an inherently lower risk of spontaneous abortion. Furthermore, the contribution that careful periconceptual medical supervision and "tender loving care" may have played in reducing the frequency of spontaneous abortion amongst the primigravidae, and in particular the infertility patients, is very difficult to assess objectively. The Norwegian follow up study of recurrent aborters (Stray-Pedersen & Stray-Pedersen 1984) documented that subsequent pregnancy outcome was successful in 86% of women receiving specialised antenatal counselling and psychological support compared to a success rate of only 33% in women given no specific care.

Despite these cautions, the results of this study have important implications for our clinical practice. It is clear that reproductive history is of prime importance in establishing the risk of pregnancy loss, for it appears that the most relevant predictive factor for spontaneous abortion is an abortion in the previous pregnancy. The risk of spontaneous abortion for primigravidae is low, and in contrast to previously held ideas, is also low for patients treated for primary infertility. Thus the outcome of the first pregnancy affects substantially the woman's entire subsequent reproductive performance.

For the woman who has miscarried recently, the customary medical counsel that "next time will be alright" needs to be revised, since this study shows that this advice could be misleading. Further studies are needed in order to identify those factors which determine whether a first pregnancy will result in success or failure, with the aim of being able to offer prophylactic preconceptual counselling to all nulligravidae.

CHAPTER 3.

THE INCIDENCE AND TIME OF DEVELOPMENT OF ANTI-PATERNAL LYMPHOCYTOTOXIC ANTIBODIES IN PREGNANCY.

The incidence and time of development of anti-paternal lymphocytotoxic antibodies in pregnancy.

3.1. Introduction.

During pregnancy, some women produce antibodies directed against paternally derived major histocompatibility antigens of the fetus (Payne & Rolfs 1958, Van Rood et al 1958). The role of these anti-paternal lymphocytotoxic antibodies (APCA) in pregnancy is unclear. Although their name might suggest that they are harmful to the fetus, paradoxically APCA have been demonstrated most frequently in the serum of multiparous women who have had successful pregnancies (Payne 1962, Jensen 1962, Doughty & Gelsthorpe 1971). APCA are usually absent from the serum of women who abort recurrently (McIntyre et al 1984, Mowbray et al 1983, Johnson et al 1984, Biddle et al 1987). This observation has led to the suggestion that recognition of the antigenic dissimilarity of the fetus might be involved in a maternal immunological adaptation required to protect the fetal allograft. Failure to achieve the appropriate immunological response may be a cause of recurrent spontaneous abortion. This hypothesis has led to the introduction of treatment in which patients with recurrent abortion who are APCA negative are immunised with whole donor blood or partner's lymphocytes (Taylor & Faulk 1981, Mowbray et al 1983).

The results obtained in the Early Pregnancy Loss Field Study (Chapter 2) suggested that the achievement of a successful pregnancy conveys a protective effect to a woman's subsequent pregnancy, and that some factor which allows pregnancy to continue successfully is lacking from women with recurrent abortion. The possibility that an immunological event occurring during the previous pregnancy offers protection against abortion in a subsequent pregnancy provides one plausible explanation for these findings. However, there is no report of the incidence and significance of positive APCA tests measured sequentially in the serum of women during pregnancy. The present study was designed to establish the natural history of APCA in successful pregnancies and spontaneous abortions, in order to determine whether serum APCA is a useful screening test with which to 1) assess a patient's prognosis for subsequent pregnancy outcome and 2) identify patients with recurrent spontaneous abortion who may be suitable for immunisation treatment.

3.2. Materials and Methods.

3.2.a. Patients.

The 338 patients participating in this part of the study were drawn from the Early Pregnancy Loss Field Study population (EPL) previously described in Chapter 2 (p 125). Of these, 145 (43%) women had responded to the radio appeal, 118 (34%) had suffered a recent miscarriage and 64 (19%) had been seen for infertility investigations (including 24 patients participating in the *in vitro* fertilisation programme, (IVF)). The remaining 11 patients (3%) had been referred because of problems in a previous pregnancy or for general medical problems.

A blood sample was obtained prior to pregnancy from all the volunteers who then notified us as soon as they became pregnant. Those patients recruited to the study after a miscarriage provided a blood sample at the time of admission to the hospital for uterine evacuation of retained products of conception. A second sample was obtained from this group of patients 2-4 weeks after their spontaneous abortion.

Study pregnancies were confirmed and dated by pelvic ultrasound. Serial blood samples were obtained from each patient at approximately 8 weeks gestation, before 12 weeks gestation (at the antenatal booking visit), at 18 weeks, 28 weeks, 32 weeks and at the time of delivery. The final maternal blood sample was drawn 4 weeks postpartum, and at this time a paternal blood sample was obtained with which to perform the lymphocytotoxicity testing.

Patients who aborted during the study pregnancy had a blood sample taken at the time of the abortion (\pm 3 days) and a further sample taken 2-4 weeks later, when a sample of paternal blood was also taken.

3.2.b. Microdroplet lymphocytotoxicity assay.

3.2.b.i. Maternal sera.

The pre-pregnancy and serial samples of serum were stored at -20°C until needed for testing against their partner's lymphocytes.

3.2.b.ii. Isolation of paternal lymphocytes.

A 5ml citrated sample of fresh peripheral whole blood was obtained from each patient's partner within 24 hours of performing the assay. 1ml of citrated blood was layered gently over an equal volume of lymphocyte separation medium (Lymphoprep, Flow Laboratories, Irvine, Scotland) in LP3

plastic microtubes and centrifuged at 1,200g for 20 minutes. The buffy coat was removed with a small quantity of supernatant plasma, washed twice with phosphate buffered saline (PBS, Oxoid Ltd, England) and centrifuged at 1,200g for a further 5 minutes. The cell pellet was resuspended in PBS to provide a final cell concentration of 1.5×10^6 cells per ml.

3.2.b.iii. Lymphocytotoxicity assay.

The microdroplet assay described by Mittal et al (1969) and standardised by the National Institutes of Health was used with the following modifications. A 64 well Terasaki microtitre plate (Falcon, Lab Tek, Naperville, U.S.A.) was covered with light paraffin oil (BDH Chemicals, Poole, Dorset, England). 1µl of cells were incubated with 1µl of the appropriate antiserum for 30 minutes at room temperature. Each maternal serum sample was tested in quadruplicate. A known anti-HLA positive serum (Kontroll-HLA, Biotest AG, Frankfurt, F.R.G.) with 100% cytolytic activity and a negative control (<5% cytolytic activity) were run in parallel. A 'blank' well on each plate contained no serum. 5 µl of rabbit complement (Buxted Rabbit Farm, Buxted, Sussex, England) were added to each well and incubated for 60 minutes at room temperature. Following incubation, 2µl of eosin solution (5% w/v) were added for 2 minutes, followed by 5 µl of 10% phosphate buffered formalin to fix the cells. The test plates were left overnight for the cells to settle before being scored under the phase contrast microscope (Nikon phase contrast, magnification x250).

The plates were read by two observers independently and the results recorded as positive when cell lysis in excess of 20% above the negative background control was present.

3.2.c. Statistical analysis.

The statistical tests used were the Chi-squared two-tailed test with Yates correction, and Fisher's exact test for samples with cell totals of less than 5. Where applicable, the Odds Ratio (OR) and the 95% Confidence Limits (CL) are given (Gardner & Altman 1986)

3.3. Results.

3.3.a. Pregnancy outcome.

Between February 1986 and September 1988, 338 women became pregnant and completed their pregnancies. The outcome of these 338

pregnancies was as follows: 283 live births, 50 spontaneous abortions, 2 ectopic gestations and 3 pregnancies which were aborted therapeutically because of fetal abnormality. Spontaneous abortion was defined according to the WHO classification (1977) as the spontaneous loss of an intrauterine pregnancy before 20 weeks gestation. The diagnosis was confirmed by pelvic ultrasound.

The 2 ectopic gestations and the 3 pregnancies which were terminated therapeutically have been excluded from further analysis. Partner's lymphocytes were not available for 8 pregnancies, post-partum serum has not been obtained from 15 of the patients and 4 women were lost to follow-up after delivery. Hence, the subsequent analyses are based on 256 live births and 50 spontaneous abortions.

3.3.b. Incidence of antipaternal cytotoxic antibody in the postpartum serum of 306 completed pregnancies.

The overall incidence of positive antipaternal cytotoxic antibody tests (APCA) in 306 completed pregnancies was 28% (86). Of the 256 women who had live births, 32% (81) had antibody present in their postpartum serum. Only 10% (5) of patients whose pregnancy ended in spontaneous abortion had positive APCA tests in their post-abortion serum sample (Table 3.1).

Table 3.1.

Incidence of pregnancies in which Anti-Paternal Cytotoxic Antibodies (APCA) were detected postpartum.

Outcome of pregnancy	Total Number of patients	No.APCA Positive (%)
Live	256	81(32) ^a
Abortion	50	5(10) ^b
Total	306	86(28)

a vs b: $\chi^2=8.65$; $p=0.003$; OR=4.2; 95% CL: 1.5-10.8

3.3.c.Time of development and cumulative frequency of APCA.

Of the 256 live births, nine patients had APCA present in their pre-pregnancy serum (Figure 3.1). All of these patients had been pregnant previously and two had received blood transfusions. All nine remained APCA positive throughout the study pregnancy. Of the 247 women with

successful study pregnancies whose pre-pregnancy serum sample contained no detectable APCA, 72 (29%) became APCA positive during the course of their pregnancy (Figure 3.1).

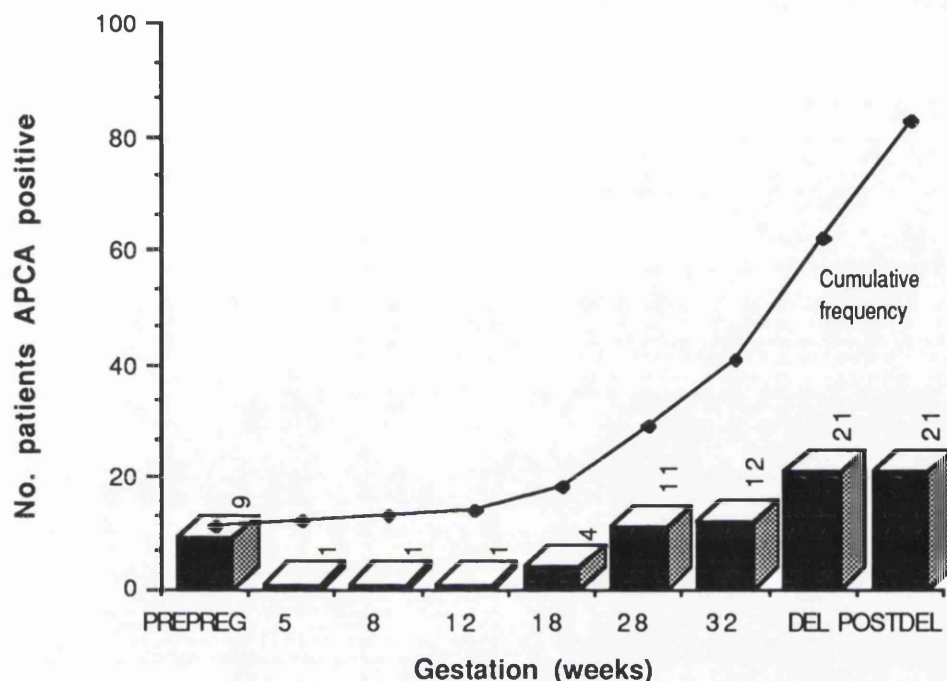


Figure 3.1. Histogram showing the incidence and time of first detection of positive anti-paternal cytotoxic antibody tests (APCA) amongst 283 women who had successful pregnancies (live births). The number of women with positive APCA tests at each gestational sampling interval is denoted above each histogram bar. The cumulative frequency of positive APCA tests is represented by the solid line (—).

One woman developed antibodies at 5 weeks, 1 at 7 weeks, 1 at 10 weeks, and 4 were first positive at 18 weeks gestation. The majority of the 72 patients who seroconverted did so during the third trimester of pregnancy, 11 at 28 weeks, 12 at 32 weeks, 21 at the time of delivery and in a further 21 patients antibodies were only detectable in the postnatal sample. Once detected, all but 2 of these 72 patients remained antibody positive throughout the remainder of their pregnancy. The first exception produced transient detectable antibodies at 32 weeks, whilst the second produced detectable levels at 18 weeks which then became undetectable again until the delivery interval. Neither patient had any evidence of an ante-partum bleed. Amongst the 50

pregnancies ending in spontaneous abortion, 5 women were found to have positive serum APCA tests (Table 1). Three of these (6%) had APCA present in their pre-pregnancy serum, whilst only 2 patients in this group (4%) developed the circulating antibodies during the study pregnancy, at 9 weeks and 10 weeks gestation respectively.

A similarly low incidence of positive APCA tests was noted amongst the 118 patients who had been recruited to the study immediately after a spontaneous abortion before 12 weeks gestation. Only 5 of the serum samples obtained at the time of the previous spontaneous abortion contained detectable APCA (4%).

3.3.d. Incidence of APCA matched for duration of gestation.

Since serum APCA appeared late in pregnancy and all the spontaneous abortions in this study group occurred before the completion of 12 weeks gestation, the incidence of positive APCA tests at 12 weeks gestation in patients whose pregnancy ended successfully were compared with those pregnancies which ended in spontaneous abortion (Table 3.2).

Table 3.2.

Number of APCA positive patients at 12 weeks gestation and subsequent outcome of their pregnancy.

Outcome of pregnancy	Total No. Patients	No. APCA Positive(%)
Live	256	12(5) ^a
Abortion	50	5(10) ^b
Total	306	17(6)

a vs b: Fisher's exact test; $p=0.12$; $\chi^2=1.35$; $p=0.25$

The incidence of positive APCA tests at this gestational interval was 10% in those pregnancies which ended in spontaneous abortion, compared to 5% in the pregnancies with live outcomes. When the patients whose pre-pregnancy serum sample contained APCA were excluded, there was no difference between the two groups in the incidence of detectable APCA by 12 weeks gestation: 4% (2/47) in the aborting group versus 1% (3/247) in the live outcome group ($\chi^2=0.74$; Fisher's exact test $p=0.19$)

3.3.e. Effect of gravidity on the incidence of APCA.

The incidence of serum APCA before and after completion of pregnancy is shown in Table 3.3, in which the 306 women have been grouped by gravidity and pregnancy outcome.

Table 3.3.

Incidence of APCA in 306 patients before and after completion of pregnancy grouped by gravidity

Pregnancy outcome	Incidence APCA	Gravidity group				Total
		1st	2nd	3rd	4th	
Live	Before	0/59	4/83	2/59	3/55	9/256
	After	20/59	24/83	17/59	20/55	81/256
Abortion	Before	0/5	0/16	1/15	2/14	3/50
	After	0/5	0/16	3/15	2/14	5/50
Total Number. APCA positive patients after completion of study pregnancy (%)		20/64	24/99	20/74	22/69	86/306
		31%	24%	27%	32%	28%

Although none of the primigravidae had APCA present in their pre-pregnancy serum sample, 34% (20/59) of those who had successful pregnancy outcomes had become positive at the completion of their pregnancy. Examination of multigravidae with successful study pregnancies suggests that, in most women, detectable APCA disappear from the serum between pregnancies. Thus only 5% of the patients (4/83) embarking on a second pregnancy had positive pre-pregnancy APCA levels whereas after completion of a successful pregnancy, 29% (24/83) of this group were APCA positive ($\chi^2 = 15.51$; $p = 0.00008$; OR=8.0; 95%CL: 2.5-29.0). Similar marked differences in the incidence of serum APCA before and after completion of a successful pregnancy were noted within gravidity group 3 ($\chi^2 = 12.30$; $p = 0.0005$; OR=11.5; 95%CL: 2.4-45.2) and gravidity group 4 ($\chi^2 = 14.07$; $p = 0.0002$; OR=9.9; 95%CL: 2.5-32.8).

There was no increase in the incidence of positive APCA patients before or after completion of pregnancy with increasing gravidity (Table 3.3). These findings in successful study pregnancies were in direct contrast to those noted for the 50 pregnancies which ended in spontaneous abortion. None of the 5 primigravidae and 16 secundigravidae who had unsuccessful pregnancies had detectable APCA before pregnancy and none of these patients developed APCA after their spontaneous abortion. Furthermore, although serum APCA was detectable in 7% of the 15 patients embarking on their third pregnancy and 14% of the 14 patients on their fourth pregnancy, there was no increase in the incidence of APCA in these women after their pregnancies had aborted spontaneously. Overall, irrespective of their gravidity grouping, only 10% of those patients who suffered an abortion were APCA positive at the completion of their study pregnancy, of whom only 4% (2/50) had produced APCA in that pregnancy. The incidence of APCA in primigravidae who had successful outcomes was significantly higher than for women of any gravidity whose pregnancy aborted ($\chi^2=7.45$; $p=0.006$; $OR=4.6$; 95%CL: 1.5-15.6).

3.3.f. Significance of a pre-pregnancy positive APCA test.

The outcome of the study pregnancy did not appear to be influenced by the presence of serum APCA prior to pregnancy. Of the 12 women who had detectable APCA in their pre-pregnancy serum sample 3 had pregnancies which ended in spontaneous abortion before 12 weeks gestation (25%) compared to 16% (47/294) in the patients who embarked on their pregnancy without detectable APCA in their serum ($\chi^2=0.18$; Fisher's exact test $p=0.3$).

Only 2 of the women with detectable APCA before pregnancy gave a history of previous blood transfusion and all 12 had had at least 1 live pregnancy in the past, although for 5 of these patients the last pregnancy had ended in spontaneous abortion. There was no difference in the mean interval between the last pregnancy and this study pregnancy in this group of 12 patients (12.2 months) when compared to the 294 women who were APCA negative at the start of their pregnancy (12.0 months).

3.3.g. Influence of past obstetric history.

The influence of past obstetric events on the incidence and time of development of APCA was examined (Table 3.4).

Table 3.4.

Influence of past reproductive history on the incidence of APCA

Patient History	No.APCA positive after pregnancy	Total No.Patients	%APCA positive after pregnancy
One abortion only	5	43	12 ^a
Only abortions in the past	11	71	16 ^c
Last pregnancy aborted	24	87	28
Primigravidae	20	64	31 ^b
Only live pregnancies	18	57	32
Last pregnancy was live	26	74	35 ^d
Previous termination of pregnancy	10	24	42

a vs b; $\chi^2=4.49$; $p=0.03$; OR=3.5; 95%CL: 1.1-11.7

b vs c; $\chi^2=3.87$; $p=0.04$; OR=2.5; 95%CL: 1.0-6.2

a vs d; $\chi^2=6.55$; $p=0.01$; OR =4.1; 95%CL: 1.3-13.6

c vs d; $\chi^2=6.36$; $p=0.01$; OR =2.9; 95%CL: 1.2-7.1

The incidence of APCA was lower in women who had suffered single or multiple episodes of spontaneous abortion than in primigravidae, women in whom all previous pregnancies had had live outcomes, women whose last pregnancy had resulted in a live child and women with a history of therapeutic termination of pregnancy. The incidence of positive APCA tests in those women whose last pregnancy had ended in spontaneous abortion (a heterogenous group including previous live births as well as previous abortions) was of the same order as other obstetric groups of women whose history included a successful pregnancy.

3.3.h. Possible factors contributing to APCA production.

A history of vaginal bleeding was noted in 68 of the 256 patients with live outcomes in this pregnancy of whom 22 (32%) were APCA positive at the completion of pregnancy. In the remaining 188 patients with no history of

overt vaginal bleeding, 59 became APCA positive (31%). For the 50 pregnancies which ended in spontaneous abortion, 39 (78%) had a history of bleeding, of whom 4 produced APCA (10%). The incidence of positive APCA tests in the 11 aborted pregnancies with no history of bleeding was similar (9%).

The use of pre-natal invasive diagnostic techniques did not appear to influence the development of APCA. Amniocentesis or chorion villus biopsy was performed in 17 pregnancies in this study but only 4% (3/81) of the pregnancies which subsequently became antibody positive had undergone a pre-natal diagnostic test, compared to 8% (14/175) of the APCA negative group ($\chi^2=1.03$; $p=0.31$). Amongst the 56 patients delivered by caesarean section, 45% (25) were APCA positive at the completion of pregnancy. However, only 12 of this group (21%) became positive at the delivery and postnatal sampling intervals, a figure similar to the incidence of 15% (30/200) found in patients who underwent vaginal delivery at the same gestational intervals ($\chi^2=0.89$; $p=0.35$).

A history of previous blood transfusion or the presence of rhesus negative blood group antibodies did not influence the likelihood of producing APCA. Thromboembolic disease, thyroid disorders and a maternal smoking history were not found more commonly in the APCA positive patients, although 5% (4) of this group suffered migrainous headaches, whereas no such history was obtained from APCA negative patients. The frequency of pre-eclampsia, premature delivery, intrauterine growth retardation and neonatal congenital abnormality was not increased or decreased within the antibody positive group.

Of 64 patients receiving treatment for infertility 34% (22) developed APCA. Although the incidence of antibodies in the 24 IVF/GIFT conceptions included in this infertility group was 50% (12), this was not significantly different from the other infertility patients ($\chi^2=3.12$; $p=0.08$) nor from the total population whose pregnancies were successful ($\chi^2=2.56$; $p=0.1$). None of these patients had received donor sperm. Amongst the IVF/GIFT pregnancies which did not develop APCA during this study pregnancy, were 2 patients who had had blood transfusions and a further 2 patients whose oocytes had been fertilised with donor sperm.

3.4. Discussion.

The results from this study confirm the findings of previous reports (Ahrons 1971a, Nymand et al 1971) that the presence of maternal serum lymphocytotoxic antibodies in pregnancy directed against paternally-derived fetal major histocompatibility antigens do not have adverse effects upon the fetus or the outcome of pregnancy. Since circulating APCA are not related to abortion (Thlikainen et al 1974, Harris & Lordon 1976, Jensen 1964) and are found most frequently in women who have successful pregnancies, it has been suggested that such antibodies are generated in most normal pregnancies (Tongio et al 1972, Jonker et al 1977) but are only detected in the serum of some women because they are fixed on the fetal tissues possessing the antigens against which they are directed (Ahrons 1971b).

However, studies recording the incidence of lymphocytotoxic antibodies against paternally derived HLA antigens in pregnancy have reported variable results ranging from 6-50% (Van Rood et al 1958, Terasaki et al 1970, Stastny 1972, Tongio et al 1977). This variability may reflect differences in assay technique (Terasaki & McClelland 1964, Engelfreit & Britten 1975) the percentage cytolytic activity chosen to represent a positive result (Doughty & Gelsthorpe 1971, Ahrons 1971b) the target antigen used (Harris & Lordon 1976, Nymand 1974) and the populations of women screened (Overweg & Engelfreit 1969, Terasaki et al 1970). Furthermore, the majority of published studies using the standardised NIH assay technique, have utilised tissue typing panels of donor lymphocytes and have not documented the presence of anti-paternal lymphocytotoxic antibodies in maternal serum at comparable gestational intervals in women of comparable parity. There have been no studies documenting the incidence and development of APCA directed against partner's lymphocytes and measured sequentially throughout pregnancy in a general population of pregnant patients whose obstetric history has been recorded accurately. Although the lymphocytotoxicity assay used in this study does not differentiate between anti-HLA antibodies and anti-idiotypic antibodies to HLA-DR receptors, it was chosen deliberately in order to allow comparisons with the studies of Mowray et al (1987).

In this study, recruits were drawn from several sources in order to achieve a representative cross-section of pregnant women. In addition the pre-pregnancy APCA status of all the recruits was documented in order to be able to assess any pregnancy-induced changes. Since the pregnancy sampling

intervals were chosen to coincide with hospital antenatal visits, a high degree of patient compliance was achieved and 91% of the completed study pregnancies could be included in the final analysis.

This study demonstrates that the incidence of positive serum APCA tests are related to the duration of pregnancy. A significantly lower incidence of APCA was noted in women whose pregnancy ended in spontaneous abortion before 12 weeks gestation (10%) when compared to women who went on to have live births (31%). However, the sequential serum sampling intervals employed illustrated clearly that the observed difference between these two groups of patients was dependant upon the gestational age that the pregnancy reached, rather than a difference between patients with successful or unsuccessful pregnancies. That the majority of patients who became APCA positive and who had live births did not develop APCA until the pregnancy had reached a gestational age of 28 weeks or more, is in agreement with the findings of Doughty and Gelsthorpe (1971) who noted the incidence of APCA at delivery to be higher than sera screened at earlier stages in pregnancy. However, this finding is in direct contrast with several other studies (Vives et al 1976, Tongio & Meyer 19077, Gelabert et al 1981) reporting a decline in the incidence of positive APCA tests during the last trimester of pregnancy. It should be noted that these were not sequential studies, but were based on single samples of blood taken from different patients at various gestations during pregnancy.

This study also suggests that gravidity alone does not influence the incidence of positive APCA tests (Table 3.3; p 147). The incidence of antibody present in primigravidae at the completion of pregnancy was no different from multigravidae. Previous studies concluding that the incidence of APCA positive sera increases with the number of previous pregnancies (Terasaki et al 1970, Stastny 1972, Nymand 1974) are difficult to interpret as they lack detailed documentation of previous pregnancy outcome.

The demonstration from this study that there are significant differences in the incidence of APCA when the previous obstetric history is taken into account, emphasizes the importance of distinguishing between successfully completed pregnancies and those that end in spontaneous abortion. The low incidence of APCA in women with a history of unsuccessful pregnancy is in agreement with previous reports (Mowbray et al 1983, McIntyre et al 1984, Johnson et al 1984). However, since the incidence of APCA after a single abortion (12%) was no different from the figure for repeated episodes of

spontaneous abortion (16%), it was not possible to agree with Gelabert's hypothesis (1981) that previous abortions sensitise the mother to paternal antigens, thereby resulting in a higher incidence of detectable APCA with an increasing number of previous abortions.

Indeed the results from this study suggest that the incidence of APCA will only be affected significantly by a pregnancy completed successfully. Primigravidae with successful pregnancy outcomes seem to have a higher incidence of APCA than women whose history includes several spontaneous abortions only, however the figure for primigravidae (31%) was the same as for women with 1,2,3 or 4 live pregnancies only (averaging 32%) and similar to that found in women whose last pregnancy had been successful (35%). Moreover, the incidence of positive APCA tests amongst women whose last pregnancy aborted is higher than for women with a history of abortions alone, since this group of women had all had a previous live birth in addition to their spontaneous abortions.

Although the outcome of previous pregnancies may affect the incidence of APCA, it does not appear to influence the time at which APCA is first detectable in a subsequent pregnancy. A previous spontaneous abortion did not lead to earlier APCA detection (Gelabert et al 1981). It is perhaps noteworthy that the highest incidence of positive APCA tests was found amongst those women whose history included a therapeutic termination of pregnancy (10/24, 42%). However the relatively small number of patients in this group (n=24) deters us from concluding that therapeutic termination may lead to immunisation.

Although some workers believe that a feto-maternal haemorrhage initiates APCA production (Jensen 1960, Terasaki et al 1970), others have found no such correlation (Nymand 1974). In this study, overt episodes of vaginal bleeding in pregnancy, prenatal invasive diagnostic procedures and mode of delivery, had no influence upon the incidence of APCA production. Furthermore, there was no increased incidence of APCA in women with a history of blood transfusion (Van Rood 1958, Jensen 1962, Doughty & Gelsthorpe 1971) Since only 2 patients developed Rhesus blood group antibodies, the reported increase in incidence of APCA in women immunised to rhesus antigens (Ahrons 1971a, Berah et al 1966) and in whom the rhesus disease was exacerbated by anti-HLA antibody production (Moulinier et al 1970) could not be confirmed by this study. A lower incidence of APCA production amongst smoking mothers (Nymand 1974) was not found in this

population, and none of the APCA positive women suffered from migrainous headaches.

The presence of APCA was not associated with obstetric complications or detrimental effects to the fetus (Ahrons 1971a, Balasch et al 1981). The frequency of premature delivery (Jensen 1964, Terasaki et al 1970), pre-eclamptic toxæmia (Scott et al 1976) or intrauterine growth retardation (Harris & Lordon 1976) was not increased in patients who were APCA positive before pregnancy or in whom APCA developed during the study pregnancy. The higher incidence of congenital abnormality in babies born to APCA positive mothers reported in two retrospective studies (Terasaki et al 1970, Naito et al 1970) was not confirmed in a subsequent study by the same authors (Sever & Terasaki 1970) nor by subsequent investigators (Ahrons 1971a, Harris & Lordon 1976) nor by this study.

That APCA are usually absent from the serum of women suffering consecutive abortions with no live delivery (idiopathic primary recurrent abortion) has led to the belief that a failure to produce circulating APCA may be used diagnostically to identify those women who are unable to mount the appropriate immune responses required to protect their fetal allograft. These patients may benefit from immunological treatment and this hypothesis has led to the introduction of immunisation treatment with whole donor blood or partner's lymphocytes (Taylor & Faulk 1981, Mowbray et al 1983, 1985). The mechanism whereby immunisation confers a beneficial effect is not understood, but is presumed to be secondary to the maternal immunological response stimulated by the infusion of allogeneic (paternal) lymphocytes.

Several studies (McIntyre et al 1984, Hofmeyer et al 1987, Beer et al 1985) have suggested that the higher incidence of positive APCA tests found in women suffering consecutive abortions following delivery of live children or stillbirths (secondary recurrent abortion) may be used to discern a further group of women with differing immunological responses to their pregnancy failures who are unlikely to benefit from immunological therapy. The results from this study suggest that the absence of APCA is unlikely to be a valid diagnostic marker for either group of patients since (1) their repeated pregnancy failures occur before the stage in gestation at which APCA are detectable, (2) APCA are only detectable in a minority of successful pregnancies, (3) detectable APCA disappear from the serum between pregnancies in most women and (4) the higher incidence of APCA in

secondarily aborting women may reflect principally their previous achievement of a third trimester pregnancy.

The success of immunisation treatment using paternal leucocyte infusions has been correlated with the induction of APCA in the serum of recurrent aborters who have negative APCA tests (Reznikoff-Etievant et al 1985). The prognosis for the subsequent pregnancy is based on the development of a positive APCA test following their infusion treatment. Patients who remain APCA negative are reported to be more likely to miscarry again, hence some workers offer booster injections (Mowbray et al 1987) or continue the infusion treatments until seroconversion has been achieved (Reznikoff-Etievant et al 1988, Alexander et al 1988, Carp et al 1988). The use of this serum marker to assess a patient's suitability for immunisation treatment and to monitor immunisation treatment response is based on the assumption that a patient who seroconverts has successfully achieved the appropriate immunological response to protect her fetal allograft, although it is recognised that APCA may be only an indirect index of the immunological response (Reznikoff-Etievant et al 1985). However, in the study presented in this thesis the presence of APCA in the serum prior to pregnancy did not offer protection from spontaneous abortion, since 25% (3/12) of the patients who fell into this category suffered a spontaneous abortion in the study pregnancy compared to 16% (47/294) of patients APCA negative prior to pregnancy.

This prospective study of the incidence and time of development of serum APCA measured sequentially before, during and after completion of pregnancy has shown that the serum sampling time is the most important determinant of the incidence of positive APCA tests. APCA are only rarely found in non-pregnancy serum and the serum of women who have suffered recurrent episodes of spontaneous abortion. A minority of women who complete a successful pregnancy develop a positive APCA test but positive APCA status usually disappears between pregnancies.

The results from this study demonstrate that the presence of detectable circulating APCA are not directly related to the success or failure of an early pregnancy and are not necessary for the maintenance of successful pregnancy.

CHAPTER 4.

PREGNANCY OUTCOME FOLLOWING IMMUNISATION TREATMENT WITH PATERNAL CELLS FOR RECURRENT ABORTION.

Pregnancy outcome following immunisation treatment with paternal cells for recurrent abortion.

4.1. Introduction.

The belief that some women who abort recurrently may have an immune deficiency which prevents them from mounting the appropriate protective response towards their fetus suggests that this cause of recurrent abortion may be remediable. Immunisation treatment using whole donor blood or partner's lymphocytes has been introduced by several centres following reports of successful pregnancy outcome in 70-80% of treated patients (Table 1.5; p 86). The mechanism whereby immunisation confers a beneficial effect has not been established, but is presumed to be secondary to the maternal immunological response stimulated by the infusion of allogeneic (paternal) lymphocytes.

In general only anti-paternal cytotoxic negative (APCA) patients are considered suitable for this form of therapy and the majority of centres offering immunisation treatment are using pre-treatment APCA negative test results as an inclusion criterion for immunisation. The production of APCA is used as a prognostic factor for subsequent pregnancy outcome, since several centres have found that patients who undergo seroconversion after therapy have improved pregnancy outcomes compared with those patients who remain APCA negative (Reznikoff-Etievant et al 1985, Mowbray et al 1985, Alexander et al 1988). Patients who remain APCA negative following their infusion treatment are reported to be more likely to miscarry again, hence some workers offer booster injections (Mowbray et al 1987) or continue the infusion treatments until seroconversion has been achieved (Alexander et al 1988, Carp et al 1988, Reznikoff-Etievant et al 1988).

The use of this serum marker to assess a patient's suitability for immunisation treatment and to monitor treatment response is based on the assumption that a patient who seroconverts has successfully achieved the appropriate immunological response to protect her fetal allograft, although it is recognised that APCA may be only an indirect index of the immunological response (Reznikoff-Etievant et al 1985). The results from the study described in Chapter 3, documenting the natural history of APCA in women with successful pregnancies and spontaneous abortions, suggested that the presence of APCA in the serum prior to pregnancy did not offer protection from spontaneous abortion.

The next part of this study was designed to assess the efficacy of paternal white cell infusion treatment for couples attending the recurrent abortion clinic in Cambridge and aimed to identify other factors in the history and investigation of patients with recurrent abortion which could be used to provide a prognosis for future pregnancy outcome.

4.2. Materials and Methods.

4.2.a. Patients.

Couples with at least 3 abortions with the same partner (primary recurrent abortion) or no more than one live birth followed by 3 consecutive pregnancy losses (secondary recurrent abortion) were referred to the Cambridge Recurrent Abortion Clinic for investigation of their recurrent pregnancy losses. The 19 recurrent aborters from the Cambridge area who were described in Chapter 2 (p 125) were included in this part of the study, but the majority of patients (202) were recruited from outside the catchment area. A full medical, social, gynaecological and obstetric history was obtained. For all previous pregnancies details including gestational age, ultrasound findings and pregnancy complications were sought together with the results of operative procedures and cytogenetic and pathological investigations of the fetus. All patients underwent a general medical and gynaecological examination.

4.2.b. Screening investigations.

The screening procedures included chromosomal analysis of both partners, hysterosalpingography for evidence of uterine body anomalies and cervical incompetence, thyroid function testing, a random blood sugar level, serological tests for toxoplasmosis, cytomegalovirus and Herpes simplex virus. Auto-antibody screening, anti-cardiolipin levels and phospholipid dependant coagulation tests (KCT) were performed on the majority of patients. All the women were tested for the presence of anti-paternal cytotoxic antibody (APCA) directed against their partners lymphocytes according to the method described in Chapter 3 (p 142). Couples considered suitable for immunisation treatment underwent additional screening investigations for blood group type, hepatitis B surface antigen and human immunodeficiency virus.

4.2.c. Immunisation procedure.

One unit (450 mls) of whole blood anticoagulated with acid citrate dextrose was obtained from the male partner using a sterile technique on the day of treatment. The paternal blood was centrifuged at 1,200g for 20 minutes. The buffy coat was removed, diluted with an equal volume of Hartmann's solution and layered gently over an equal volume of lymphocyte separation medium (Lymphoprep, Flow Laboratories, Irvine, Scotland) in four 30ml polystyrene tubes (Sterilin Ltd, Teddington). These were centrifuged for 20 minutes at 1,200g, following which the lymphocyte layer was removed and washed three times with Hartmann's solution. The cells were finally suspended in 5mls of isotonic saline. Three mls of this cell suspension were injected intravenously into the women, and 0.5mls were injected into each of 2 intradermal and 2 subcutaneous sites on the forearm, later that day. Those women with a Rhesus negative blood group who received a white cell infusion from a Rhesus positive partner were given an intramuscular injection of 500iu of anti-D immunoglobulin.

A blood sample was obtained from both partners 6 weeks after immunisation treatment. The women's serum was tested against her partners cells for the presence of APCA as previously described (Chapter 3, p 143).

4.2.d. Follow up.

All patients referred to the clinic were contacted at six monthly intervals following their first attendance. Both immunised and non-immunised patients were asked to notify the clinic as soon as they became pregnant. All the patients were invited to reattend the clinic following the first missed period for confirmation of their pregnancy by serum hCG testing and pelvic ultrasound (see Chapter 2; p 123). Serial ultrasound monitoring was performed every fortnight in those patients who were able to attend the clinic regularly. Patients developing symptoms of threatened abortion were immediately submitted to an ultrasound examination and any pregnancy which ended in spontaneous abortion was categorised using the ultrasound criteria described in Chapter 2 (p 124) as complete, incomplete, missed, anembryonic and fetal spontaneous abortions. Whenever possible patients who required uterine evacuation of products of conception had this procedure performed in Cambridge in order to submit the fetal tissue to cytogenetic analysis.

Arrangements were made with the patient's local hospital to monitor the progress of all continuing pregnancies throughout the antenatal period. Details of the pregnancy were completed when the patients recontacted the clinic following delivery.

4.2.e. Statistical analysis.

The statistical tests used were the Chi-squared two-tailed test with Yates correction and 2 x 3 contingency tables. Fisher's exact probability testing was applied when cells contained less than 5 samples. Where applicable, the Odds Ratio (OR) and 95% Confidence Limits (CL) are given (Gardner & Altman 1986).

4.3. Results.

4.3.a. Recruits.

Between February 1986 and December 1988 a total of 221 couples who had experienced recurrent episodes of spontaneous abortion (RSA) were referred to the Cambridge Recurrent Abortion Clinic. There were 129 couples with a history of primary recurrent abortion and 92 couples with secondary recurrent abortion.

The mean maternal age (mean \pm S.D) of the total study group was 31.5 years (± 5.2). The mean maternal age of the primary aborters was 30.9 years (± 5.4) and that of the secondary aborters 32.4 years (± 5.1). The women had had between three and eight spontaneous abortions with their current partner. A history of a previous pregnancy in which fetal heart activity had been documented by pelvic ultrasound and which subsequently ended in spontaneous abortion was noted in 39% (89) of the total study group. The incidence of previous pregnancies ending in fetal/embryonic death was similar in primary (40%, 52/129) and secondary aborters (38%; 35/92). A history of a family member who had experienced 2 or more spontaneous abortions was noted in 36% (80) of the study group, and was not different in primary and secondary aborters. A medical history of asthma, hay-fever or eczema requiring medical treatment was elicited from 12% (15/129) of primary aborting women and 17% (16/92) secondary aborters ($\chi^2 = 1.04$; $p = 0.31$).

A period of relative infertility, defined as failure to conceive after 12 months of unprotected intercourse, was noted in the obstetric history of 29% (64/221) of the patients. Of these 64 patients, 48 (75%) had required subfertility

treatment for one or more of their previous pregnancies, the majority (40) having received clomiphene medication. Hence 22% of the overall study group had undergone treatment for subfertility. The incidence of relative infertility among primary aborters was 33% (43/129) which is not different statistically from the incidence of 23% (21/92) noted for the secondary aborting couples ($\chi^2=2.39$; $p=0.1$).

4.3.b. Investigations.

The positive test results from the screening procedures performed on the patients are outlined in Table 4.1.

Table 4.1.

Incidence of positive screening tests in patients with recurrent abortion.

		No. positive tests	No. patients tested	Percentage positive %
Genetic	Balanced translocation	9 **	221	4%
	Mosaic cells, inversions etc	13	221	6%
Anatomical	Uterine anomalies	18	119	15%
	Cervical incompetence	2 *	119	2%
Infection	Toxoplasma	5 *	185	3%
Auto-antibodies	Cardiolipin	6 *	185	3%
	Antinuclear	2 *	185	1%
	Thyroid	2	185	1%
	Smooth muscle	4	185	2%
	Prolonged KCT	4 *	185	2%
Anti-Paternal Antibody	Cytotoxic	10 *	221	5%

* Positive test results excluding patient from immunisation treatment. No patients were found to have thyroid function disorders or abnormal glucose tolerance testing.

In addition to the identification of 9 balanced reciprocal translocation carriers, 13 patients were found to have a pericentric inversion, deletion,

satellite chromosome or a number of mosaic cells in their peripheral blood karyotypes. These 13 patients were referred for genetic counselling but none of these genetic variants were considered to be of clinical significance in the aetiology of their recurrent pregnancy losses.

There were 18 uterine body anomalies identified radiographically, 2 unicornuate uteri, 9 bicornuate uteri, 3 partially septate uteri and 4 cases of an isolated small uterine fibroid. The significance of these findings were uncertain since none of these patients gave a history of late "fetal" abortion. The obstetric histories of the 2 patients in whom a diagnosis of cervical incompetence was made suggested that the cervical anomaly might have contributed to their previous pregnancy losses.

The presence of a raised titre of toxoplasma antibodies in 5 women was the only evidence found to implicate an infectious aetiology for abortion in the population studied. The presence of anti-nuclear antibodies, anti-cardiolipin antibodies or a prolonged phospholipid dependant coagulation test were all considered to be serological evidence of an underlying auto-immune disorder and a contra-indication to immunisation treatment. Of the 10 couples with positive APCA tests 4 (4%) were noted to be primary aborters and 6 (8%) were secondary aborters (4/129 vs. 6/75; Fisher's exact testing $p=0.11$). All 10 patients were excluded from the treatment group. There were no patients with test results suggestive of thyroid dysfunction or diabetes mellitus.

4.3.c. Pregnancy outcome.

Of the 221 couples referred to the clinic, 144 (65%) became pregnant and 95 (66%) of these pregnancies had a successful outcome.

4.3.c.i. Immunisation patients.

A total of 146 couples underwent immunisation treatment and of this group 102 (70%) became pregnant. The outcome of these 102 subsequent pregnancies is shown in Table 4.2.

Amongst the primary RSA group 66% of the immunised patients conceived compared to 78% of the secondary aborters ($\chi^2=1.56$; $p=0.21$). There was no significant difference in the percentage pregnancy success rate between primary and secondary aborting couples.

Table 4.2.

Pregnancy outcome after immunisation treatment.

	No. patients immunised	Pregnancies	Abortions	Live births	%Success
Primary RSA	97	64	24	40	63%
Secondary RSA	49	38	12	26	68%
Total	146	102	36	66	65%

4.3.c.ii. Non-immunised patients.

A further 75 couples did not undergo immunisation treatment for the following reasons. Eighteen patients became pregnant before their investigations had been completed and 19 couples declined treatment. In 28 couples, a positive result from the screening investigations were considered to have contributed to the recurrent pregnancy losses and a further 10 couples were found to be APCA positive (highlighted in Table 4.1 by *). None of these patients received any other form of treatment before or during their subsequent pregnancies. Of the total 75 patients in this group, 42 patients (56%) became pregnant. The clinical outcome of these pregnancies is shown in Table 4.3. There was no difference in the pregnancy success rate between primary and secondary aborters in this non-immunised group.

Table 4.3

Pregnancy outcome in non-immunised patients.

	No. of patients	Pregnancies	Abortions	Live births	%Success
Already pregnant	18	18	4	14	78%
APCA positive	10	8	2	6	75%
Declined	19	1	1	0	0
Abnormality detected	28	15	6	9	60%
Total	75	42	13	29	69%

Only one pregnancy was identified amongst the 19 couples who refused immunisation treatment and this pregnancy ended in a spontaneous abortion. In this group 9 couples are still trying to conceive, 4 couples have been lost to follow up and a further 5 have discontinued attempts to become pregnant. Of the 28 couples with possible causes for their previous pregnancy losses, 15 declared a pregnancy (54%). Although the pregnancy success rate in this group was lower than for other categories of non-immunised patients studied this difference was not statistically significant (9/15 vs. 7/27; $\chi^2=3.41$; $p=0.06$; OR=4.3; 95% CL:0.9-20.8). The pregnancy outcome for the different subgroups of patients with "detectable abnormality" varied and are shown in Table 4.4.

Table 4.4.

Pregnancy outcome in patients with abnormal test results.

	No. of patients	Pregnancies	Abortions	Live births	%Success
Balanced translocation	9	4	1	3	75%
Cervical incompetence	2	1	0	1	100%
Infection	5	3	2	1	33%
Cardiolipin	6	3	1	2	66%
Antinuclear Antibodies	2	1	0	1	100%
Raised KCT	4	3	2	1	33%
Total	28	15	6	9	60%

4.3.d. Characteristics of the abortuses.

Of the 49 pregnancies ending in spontaneous abortion, ultrasound examination permitted classification of the type of abortion (for definition of these terms refer to Chapter 2.2 as follows: - 8 Complete (16%), 3 Incomplete

(6%), 9 Anembryonic (18%), 11 Missed (22%) and 18 Fetal (37%). Although 78% (14) of the fetal abortions occurred in women with a history of primary recurrent abortion, the incidence of fetal abortions among primary and secondary aborters was not significantly different (14/29 vs. 4/20; $\chi^2=2.56$; $p=0.1$). Although there were no cases of anembryonic or missed abortions in the non-immunised group (Table 4.5), no statistically significant differences between the types of abortion in immunised and non-immunised women were evident.

Table 4.5

Ultrasound classification of the 49 abortions.

	Immunised		Non-immunised		Total(%)
	1 ^o RSA	2 ^o RSA	1 ^o RSA	2 ^o RSA	
Fetal	11	1	3	3	18(37%)
Missed	8	3	0	0	11(22%)
Anembryonic	3	6	0	0	9(18%)
Complete	2	2	0	4	8(16%)
Incomplete	0	0	2	1	3(6%)
Total	24	12	5	8	49(100%)

Only 27% (13) of the abortions had occurred by the completion of the eighth gestational week, 61% (30) of the pregnancies ended between 9 to 12 completed weeks and 12% (6) aborted after 13 weeks. The gestational age at which the 49 abortions occurred are shown in Figure 4.1.

Only one of these second trimester pregnancy losses occurred after 20 weeks gestation - at 22 weeks. Karyotyping of abortus material was attempted in 32 of the 49 cases of abortion (65%) and was successful in 21 cases (66%). The culture success rate for the total group was therefore 43% (21/49). Most of the culture failures occurred in products of conception obtained after a diagnosis of missed abortion or anembryonic pregnancy had been made. Products of conception were available for 15 of the 18 fetal abortions, and in 14 cases were successfully karyotyped. Only one chromosomal abnormality was detected, a case of trisomy 22 in a 38 year old woman who had undergone immunisation treatment. Of the 13 fetal abortions with normal chromosomal complements, 8 were male and 5 were female. Overall, there were 3 chromosome

abnormalities detected among the 49 abortions in this study. In addition to the trisomy 22 fetus already mentioned 2 unbalanced translocations were identified.

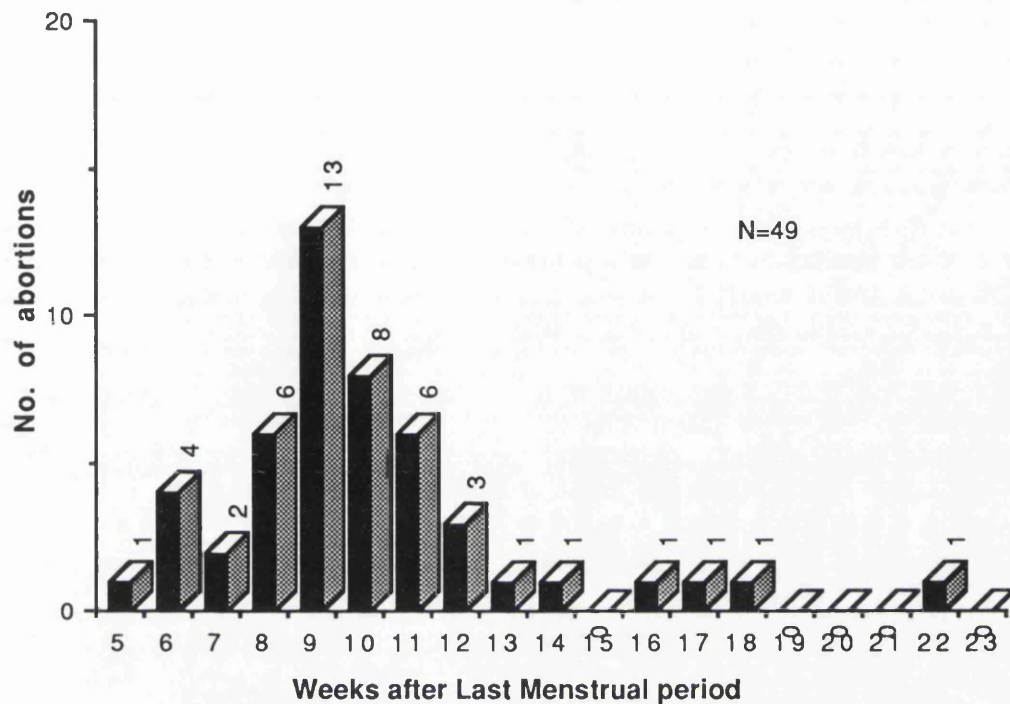


Figure 4.1. Gestational age at abortion of 49 pregnancies

4.3.e. APCA conversion rate.

All patients who received immunisation (n=146) were APCA negative prior to treatment. Seroconversion to APCA positive status 6 weeks after treatment was noted in 81 (56%) patients of whom 53 (65%) subsequently became pregnant. Of the 65 patients who remained APCA negative after immunisation 49 (75%) declared a pregnancy. The APCA seroconversion rate did not influence the pregnancy rate or the time interval to conception (Table 4.6).

Sixty-one of the total 102 pregnancies were conceived within 12 weeks of their immunisation treatment, of whom 32 were APCA positive and 29 were APCA negative. Similarly, there was no difference in the seroconversion rate among the 41 women who became pregnant 12 weeks or more after immunisation treatment, 21 were APCA positive and 20 were APCA negative ($\chi^2=0.063$; $p=0.9$).

Table 4.6

Immunisation to pregnancy interval and APCA status.

No. of pregnancies	Immunisation to pregnancy interval		Total No. of pregnancies
	≤ 12 wks	≥ 12 wks	
APCA positive	32	21	53
APCA negative	29	20	49
Total	61	41	102

Of the 66 live births following immunisation treatment (Table 4.7), 45 were to women who had become pregnant within 12 weeks of their treatment and 21 of the pregnancies had been conceived 12 or more weeks after immunisation treatment ($\chi^2=16.03$; $p=0.00006$; $OR=4.6$; 95% CL: 2.1-10.3).

Table 4.7

APCA results 6 weeks post immunisation treatment and pregnancy outcome.

APCA status		Pregnancy outcome		Total No. Pregnancies
		Live births	Abortions	
≤ 12 wks	APCA positive	24	8	32
	APCA negative	21	8	29
≥ 12 wks	APCA positive	13	8	21
	APCA negative	8	12	20
Total No. pregnancies		66	36	102
No. APCA positive		37	16	81
(%)		56% ^a	44% ^b	56%

a vs b $\chi^2=0.84$. $p=0.36$

However, this significantly improved pregnancy outcome in women who conceived promptly after their immunisation treatment was not related to post treatment APCA status (Table 4.7). Of the pregnancies which occurred within 12 weeks, 24 of the 45 live births and 8 of the 16 abortions were APCA positive ($\chi^2=1.19$; $p=0.23$). Similarly there was no correlation between pregnancy outcome and positive APCA status among the pregnancies conceived 12 weeks or more following immunisation (13/21 live births vs. 8/20 abortions; $\chi^2=0.004$; $p=0.95$). There was no significant difference in the

rate of seroconversion between primary and secondary aborters or their pregnancy outcomes (data not shown).

4.3.f. Influence of infertility.

The pregnancy rate in recurrent aborters with a history of infertility who underwent immunisation treatment was 27% (27/102) compared to 17% (7/42) in the non-immunised group, but this difference was not statistically significant ($\chi^2=1.09$; $p=0.29$). However, pregnancy outcome after immunisation treatment did appear to be influenced significantly by a history of previous infertility. Of the 36 women who suffered a further spontaneous abortion after immunisation, 14 (39%) had had a period of relative infertility in the past, whereas only 13 of the 66 women (20%) who had successful pregnancies after immunisation had experienced subfertility previously ($\chi^2=3.83$, $p=0.05$; O.R. =2.7; 95% CL 0.99-7.4). Furthermore, this difference was due entirely to the poor pregnancy outcome in those women with a combined history of secondary recurrent abortion and infertility (Aborted again 7/12; Successful pregnancies 3/26; $\chi^2=7.01$; $p=0.008$; Fisher's exact test $p=0.005$), since the pregnancy outcome among primary aborters with a history of infertility was not different (Aborted again 7/24; successful pregnancy 10/40; $\chi^2=0.005$; $p=0.9$; Fisher's exact test $p=0.47$).

The interval in weeks between immunisation and conception was calculated for the 102 study pregnancies and noted to be approximately doubled in those patients who had a spontaneous abortion (Mean 23.4 weeks) compared to those patients who had a successful pregnancy (Mean 13.8 weeks). The pregnancy success rate for women who became pregnant within 12 weeks of immunisation treatment was 74% (45/61), whereas pregnancies conceived 12 to 28 weeks after treatment had a 55% (12/22) chance of being successful and the figure dropped to 51% (9/19) when the immunisation to conception interval was greater than 28 weeks ($\chi^2=5.69$; $p=0.05$). The incidence of successful pregnancy outcomes in pregnancies conceived before and after 12 weeks from immunisation treatment were also significantly different (45/61 vs. 21/41; $\chi^2=4.52$; $p=0.03$; OR=2.7; 95% CL: 1.1-6.8).

By contrast, pregnancy outcome was not affected by a history of relative infertility in the non-immunised group of patients. A history of relative infertility was noted in 5 of the 29 couples who had successful pregnancies (17%) and 2 of 13 couples whose pregnancy aborted (15%). Interestingly the number of primary and secondary aborters with infertility histories were

distributed differently in the immunised and non-immunised patient groups. The incidence of infertility among the primary aborters was 31% (5/16) which is significantly higher than the incidence of 8% (2/26) noted for the non-immunised secondary aborters (Fisher's exact testing; $p=0.05$). Moreover, in those non-immunised women who had successful pregnancies, a history of infertility was noted for 36% (4/11) of primary aborters but only 6% (1/18) of secondary aborters (Fisher's exact testing; $p=0.054$).

4.3.g. Influence of a family history of abortion.

The overall pregnancy rate among couples with a history of 2 or more abortions in another family member was 63% (50/80). There were no differences in the pregnancy rate between primary aborters (31/48, 65%) and secondary aborters (19/32, 59%), but among the 62 couples who underwent immunisation treatment 74% (46) became pregnant which is significantly higher than the 22% (4/18) conception rate noted for non-immunised patients ($\chi^2=13.9$; $p=0.0002$; OR=10.1; 95% CL: 2.6-43.0). Furthermore, 34 of the 46 pregnancies in the immunised group were successful whereas all 4 of the pregnancies conceived in the non-immunised group ended in spontaneous abortion (Fisher's exact testing $p=0.008$).

4.3.h. Birth weight and sex ratio.

The median birth weight of the 66 babies born after immunisation treatment was 3.25 kg (mean 3.1 kg; SD ± 0.73 ; SE ± 0.1). Six of these pregnancies (9%) ended before 35 completed weeks of gestation, of which 2 pregnancies were complicated by clinical and ultrasound evidence of intra uterine growth retardation during the antenatal period. The mean birth weight of the 60 babies born after 35 weeks gestation was 3.35 Kg.

The median birth weight of the 29 babies delivered to women who had not undergone immunisation treatment was 3.22 kg (mean 3.2 Kg; SD ± 0.51 ; SE ± 0.12) and in this group the incidence of pre-term labour was 10% ($n=3$). No objective evidence of growth retardation was identified in any of these pregnancies, the mean birth weight of the babies delivered after 35 weeks completed gestation was 3.25 Kg. No significant differences in birth weight between the babies born to women with a history of primary abortion and secondary recurrent abortion were noted in either the immunised or non-immunised groups.

The male:female sex ratio among babies born to immunised mothers with a history of primary recurrent abortion was 1 : 2.1 (13 males: 27 females), but this difference was not significant statistically (Fisher's exact testing $p=0.16$). No differences in the sex ratio of the babies born in any of the other subgroups of immunised and non-immunised patients were evident.

There were no congenital abnormalities reported in the babies born to immunised mothers. One woman with a balanced translocation in the non-immunised group delivered a baby with a large diaphragmatic hernia and partial small bowel atresia who died within 24 hours of birth. This baby had a normal female chromosome complement diagnosed during the ante-natal period by amniocentesis and confirmed post-natally. Ultrasound screening had noted the structural abnormalities but the parents had decided against termination of the pregnancy.

4.5. Discussion.

In this prospective study of recurrent spontaneous abortion a successful pregnancy outcome was observed in 66% (95/144) of couples. This finding agrees with other prospective reports which have concluded that the majority of couples with a history of three or more abortions will have a subsequent successful pregnancy (Tho et al 1979, Harger 1983, Stray-Pedersen 1984) and that the poor prognosis predicted from the theoretical studies of Malpas (1938) and Eastman (1946) are unfounded (Speert 1954, Glass & Golbus 1978, Rock & Zacur 1983).

In this study the pregnancy outcome in couples with a history of primary and secondary recurrent abortion was not different, which may reflect the fact that the frequency of detectable abnormalities in this study population was not higher among the group of primary aborters, a finding in direct contrast to some studies (Harger et al 1983, Stray-Pedersen 1984). Furthermore, the incidence of pregnancy loss was not increased significantly amongst patients with a detectable abnormality (Tho et al 1979, Rock & Zacur 1983, Harger et al 1983).

A positive screening investigation was noted in 65 (29%) of the couples studied and in only 28 (13%) of cases was the abnormal result considered to be significant in the aetiology of the pregnancy losses. The percentage incidence of possible aetiologic factors was considerably lower in this study when compared to those cited in Table 1.7 (p 117). The 4% incidence of balanced translocation carriers is in agreement with previous reports that have investigated couples with repeated abortions only (Simpson 1981, Stray-Pedersen 1984) and have not included individuals with a history of fetal malformation (Byrd et al 1977) or multifactorial abnormalities (Tho et al 1979). The incidence of chromosomal variants (6%) cited in Table 4.1 (p 161) are similar to previously published figures (Harger et al 1983) but were not considered to be significant in the aetiology of the abortions suffered by this population.

In this study a structural abnormality of the uterine body or cervix was seen in 17% (20) of the 119 hysterosalpingograms performed, an incidence similar to that reported by Tho et al (1979). Since the significance of such abnormalities in the aetiology of recurrent pregnancy loss is questionable (Glass & Golbus 1978) and the results of surgical correction remain unproven (Bennett 1987) only those 2 patients in whom a diagnosis of cervical

incompetence was made were excluded from the treatment group on the grounds that they had demonstrated a possible cause for their pregnancy losses. The pregnancy outcome in this group of patients did not differ from the rest of the population (data not shown). It is regrettable that the infectious screening employed did not include cervical and endometrial culture techniques, since several studies have reported high successful pregnancy rates after identification and treatment of toxoplasma and mycoplasma infections (Harger et al 1983, Stray-Pedersen 1984).

The number of patients with raised anti-cardiolipin antibody levels and prolonged coagulation testing (KCT) in this study are small, but the relatively poor subsequent pregnancy outcome noted in these patients suggests that these investigations are useful screening procedures to identify couples at high risk of further pregnancy losses and in whom treatment with low dose steroids and aspirin should be considered (Lubbe & Liggins 1988). In agreement with several previous reports the results from this study lead to the conclusion that thyroid function testing and estimation of random blood glucose levels is of no help in the investigation of recurrent abortion (Crane 1976, Harger et al 1983, Glass & Golbus 1978, Rock & Zacur 1983).

The use of early pregnancy ultrasound surveillance was an important factor contributing to the high incidence of successful chromosomal analysis achieved by this study, since many of the pregnancies which ended in abortion could be promptly identified and the products of conception obtained for examination. A comparison of Figure 2.1 (p 126) and Figure 4.1 (p 166), which document the gestational age at abortion of the study populations described in Chapters 2 and 4 respectively, demonstrate that women with sporadic episodes of abortion lose their pregnancies at earlier gestational ages than women who suffer recurrent abortion. Although the study of Strobino et al (1986) demonstrated that recurrent aborters have a tendency to abort chromosomally normal fetuses at later stages in gestation than sporadic aborters, there have been no reports documenting the incidence of "fetal" abortions in a population of recurrent aborters using the ultrasound criteria used in this study.

The overall pregnancy success rate for patients undergoing immunisation treatment with their partner's white blood cells was 65% in this study, which is lower than many reported series (Table 1.5; p 86). The possibility that differences in patient selection and immunisation technique were responsible for this difference warrant discussion.

This study group included a high percentage of secondary aborters (42%) and women with a history of relative infertility (29%). In contrast to those reports suggesting that women with a history of secondary recurrent abortion are less likely to benefit from immunisation treatment (Beer et al 1985, Smith & Cowchock 1988, McIntyre et al 1986) no difference in overall pregnancy outcome between primary and secondary recurrent aborters was noted in this study. However the finding in this study that a history of infertility has an influence upon pregnancy outcome after immunisation treatment has not been reported previously, and since this effect appeared to be concentrated within those secondary aborters who also had a history of relative infertility, the inclusion of a large number of secondary aborters may have influenced the results significantly.

The immunisation technique used was based on the protocol reported by Mowbray et al (1985) and no modifications were introduced. However the incidence of post treatment seroconversion to positive APCA status was lower in this study (56%) than in Mowbray's series (75%) which may have influenced the results since the development of APCA in serum after immunisation treatment has been correlated with improved prognosis for future pregnancy outcome (Mowbray et al 1987, Reznikoff-Etievant et al 1985, Carp et al 1988). Nonetheless, in this study the outcome of the next pregnancy did not appear to be related to the presence or absence of APCA following immunisation treatment (Table 4.7; p 167).

The deleterious effect of a history of previous infertility on the overall outcome of pregnancy noted in this study group was confined to those patients who had undergone immunisation treatment. Furthermore, analysis of the immunisation to conception intervals demonstrated that the chance of a successful pregnancy after immunisation averaged 76% when conceived within 12 weeks of treatment, but that after this time interval the pregnancy success rate was significantly lower. Although other authors have reported improved pregnancy outcome in patients who conceive promptly after their immunisation treatment (Mowbray et al 1987, Carp et al 1988), the finding has been interpreted as evidence that the immunisation treatment induces the production of factors which protect the subsequent pregnancy (possibly APCA, although this may be an indirect measure of the effect) and that this protection is maximal for a relatively short period of time.

The data from this study suggests that there is an alternative explanation for these observations. Not only does the patient with relative

infertility have a poorer prognosis for pregnancy outcome based on the history alone, but since this adverse factor tends to persist in subsequent pregnancies, these patients will take longer to become pregnant after their immunisation treatment irrespective of whether the immunisation treatment has induced protective factors. Conversely, those patients without conception difficulties have an inherently better prognosis for their next pregnancy, and since they will tend to become pregnant soon after treatment, there will ~~be~~ appear to be an association between improved pregnancy outcome in those pregnancies conceived soon after immunisation treatment. This argument gains support from 2 further observations made in this study. Firstly, that the development of APCA after treatment was not correlated with a higher incidence of subsequent successful pregnancy outcome. Secondly, the pregnancy success rate among those patients who did not receive immunisation treatment because they became pregnant before their screening investigations were completed was 78%, which is higher than the incidence found in any of the other sub groups of non-immunised or immunised patients.

The lack of an appropriate randomised control group of patients who did not receive immunisation treatment is a major criticism of this study and one which prevents definitive conclusions as to the efficacy or otherwise of immunisation treatment with paternal cells for couples with recurrent spontaneous abortion. Nonetheless, the inclusion of patients with detectable causes for their pregnancy losses together with the observation that the overall pregnancy outcome in this non-immunised group was not different from the immunised group raises some important questions.

The possibility that the non-immunised group was biased towards patients with an inherently better prognosis for future pregnancies demands consideration, since nearly half (18/42) of the pregnancies in this group were to couples who became pregnant before their investigations had been completed, and as mentioned above the high pregnancy success rate in this group may have been secondary to the clustering of patients with a low incidence of relative infertility in this group. Furthermore, the group of patients who declined immunisation treatment included a high percentage of the total number of study drop-outs. The pregnancy outcome of the 4 couples lost to follow up remains unknown and all 5 of the patients who discontinued attempts to become pregnant did so on the grounds of advancing age and a history containing multiple episodes of abortion. If subsequent pregnancies in

these women had occurred they would have been expected to have an extremely poor prognosis.

The possibility that the pregnancy outcome amongst the non-immunised patients was due to the "tender loving care" they received before and during the early stages of their pregnancies is impossible to assess objectively in this study. However the results of the frequently cited study by the Stray-Pedersens (1984) demonstrated that psychological factors made a very significant contribution to the pregnancy outcome in their population of recurrently aborting couples. Since the pregnancy outcome in immunised and non-immunised patients was not different in this study in which all patients were offered regular review including serial ultrasound scans to monitor their progress during early pregnancy, the suggestion that tender loving care played an important role for this study group as well, cannot be ignored. This possible interpretation of the results together with the finding that a majority of patients suffering recurrent episodes of spontaneous abortion are capable of a successful pregnancy raises the question of whether the implementation of any treatment programme for a condition with an inherently high spontaneous resolution rate is justified.

Although no complications secondary to treatment were encountered in this study the possibility that immunisation treatment may lead to a higher incidence of growth retardation (Menge & Beer 1985) and maternal complications such as post treatment transfusion reactions (Hofmeyer et al 1987) have been reported. Since no ectopic gestations occurred in this study it is not possible to comment on the increased incidence of this early pregnancy complication in immunised patients (Mowbray J.F and Unander A.M personal communications).

The results from this study demonstrate that recurrent spontaneous abortion is a distinct clinical entity. Patients who suffer recurrent episodes of abortion do appear to have characteristics distinguishing them from patients who have experienced sporadic spontaneous abortion. In addition to the cytogenetic and gestational characteristics of their abortions mentioned earlier, this study documented an association with a family history of abortion and found that a history of relative infertility together with a delay in conception prior to the studied pregnancy had a significant influence on pregnancy outcome. Since knowledge of these two factors are invariably available when a couple with a history of recurrent abortion present for investigation, these associations should prove useful in the assessment of their risk of future

pregnancy loss and identifies avenues for future research and treatment of recurrent abortion.

CHAPTER 5.

THE ASSOCIATION BETWEEN HIGH LUTEINISING HORMONE CONCENTRATIONS AND SPONTANEOUS ABORTION.

The association between high luteinising hormone concentrations and spontaneous abortion.

5.1. Introduction.

There have been several recent reports of an association between high follicular phase luteinising hormone (LH) concentrations and pregnancy loss in patients undergoing treatment for infertility. In women undergoing ovarian stimulation with exogenous gonadotrophin stimulated cycles for in-vitro fertilisation and embryo transfer (Stanger & Yovich 1985, Howles et al 1986) and those having treatment with luteinising hormone releasing hormone (LHRH) for anovulatory infertility (Homburg et al 1988), high LH concentrations were associated with both failure to conceive and a high rate of pregnancy loss.

One explanation for these findings is that exposure of the ovaries to high LH concentrations during the phase of follicular growth may be deleterious to the developing oocyte (Jacobs et al 1987). Raised plasma concentrations of LH are characteristic of patients with polycystic ovary syndrome (PCOS). Recent ultrasound studies of ovarian morphology have demonstrated that the prevalence of PCOS in a population of normal female volunteers of reproductive age is 23% (Polson et al 1988) and that the figure among women with a history of recurrent abortion may be as high as 82% (Sagle et al 1988).

However, there have been no studies of serum LH concentrations and subsequent pregnancy outcome in spontaneous cycles. This prospective study was designed to investigate the relationship between follicular phase LH concentrations in women of proven fertility and the outcome of pregnancy.

5.2. Materials and Methods.

5.2.a. Patients.

The patients participating in this part of the study were drawn from the populations described in Chapter 2 (p 125) and Chapter 4 (p 158). A total of 193 women who were planning to become pregnant were included. One hundred and thirty six women were recruited to the study following a miscarriage and the remaining 57 joined the study in response to the radio and poster campaign. The study population included women who were nulliparous (n=26), women who had had one miscarriage (n=24), two miscarriages (n=24) previously or three or more miscarriages (n=30). A further 89 women had had

pregnancies continuing beyond 28 weeks gestation, of whom 72 had also had one (n=20) or more (n=50) miscarriages. The mean age of the women was 30.7 years (range 21 to 43 years).

5.2.b. Methods.

A full medical, surgical, obstetric and gynaecological history was taken. All patients had a menstrual cycle length of more than 24 and less than 40 days. A single blood sample was obtained in the early follicular phase from all women. Samples were obtained from 169 patients on days 7-9, from 16 patients on days 5 or 6, and from 8 patients on days 3 or 4 of their menstrual cycle. The serum was frozen at -20°C and analysed for LH in 3 batches. A radioimmunoassay (RIA) using polyclonal anti-LH antiserum F87 and standard LH IRP 68/40 was employed. The assay bias varied from +2.1 to -6% (National External Quality Control Assessment Scheme), with a precision profile coefficient of variance of 4.3 - 7.5% in the region of 10 IU/L. An LH concentration of greater than 10iu/l was used to define the "high LH" group (Eden et al 1988, 1989; Conway et al 1988; Polson et al 1988).

Patients were requested to contact the clinic as soon as a pregnancy occurred. Pregnancy was confirmed by ultrasound evidence of an intrauterine gestation sac and serum β -hCG testing. "Biochemical" pregnancies were not included in the analysis. The pregnancy outcome was recorded as a miscarriage if non-viability was demonstrated on ultrasound scanning of the uterus or if the pregnancy ended spontaneously before 20 completed weeks of gestation (WHO 1977). Successful pregnancy was defined as the delivery of a live infant or a pregnancy continuing beyond 28 weeks gestation whether live or stillborn. Patients who had not notified the clinic of a pregnancy within 12 months were contacted by telephone or letter at 6 monthly intervals.

5.2.c. Statistical analysis.

Statistical analyses were performed using the Chi-squared two-tailed test with Yates' correction and Fisher's exact probability testing.

5.3. Results.

The distribution of pre-pregnancy LH concentrations in the total population is shown in Figure 5.1. in which pre-pregnancy LH concentrations in women who became pregnant and those who did not conceive during the

study period are compared. The majority of patients who became pregnant had LH concentrations below 10 iu/l.

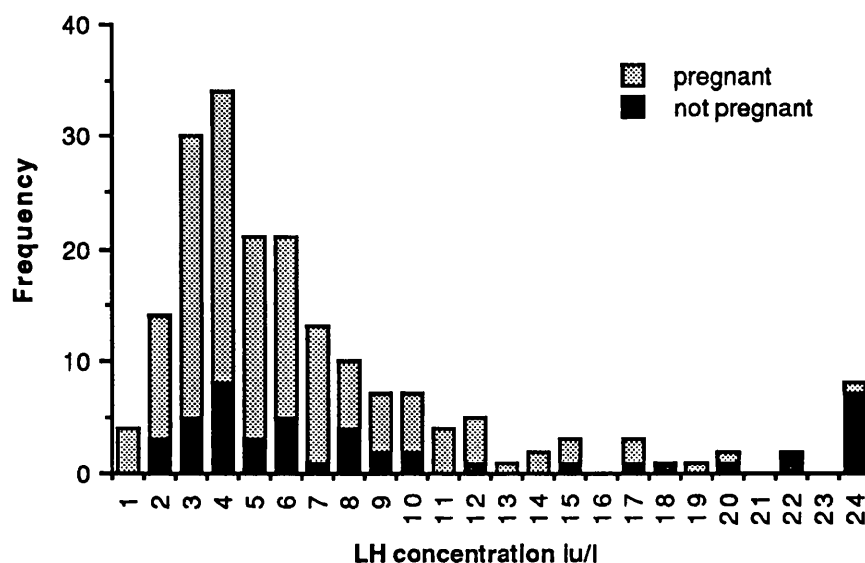


Figure 5.1. Distribution of pre-pregnancy luteinising hormone (LH) concentrations in 193 women of whom 146 became pregnant and 47 did not become pregnant.

Of the 193 women recruited to the study, 146 became pregnant, of whom 33 miscarried (17%). Of these 33 pregnancy losses, 31 occurred before 12 completed weeks of gestation. Of the 193 study patients, 147 (76%) had LH concentrations of less than 10 iu/l, of whom 118 (80%) conceived (Table 5.1). Forty six women (24%) had LH concentrations greater than 10 iu/l ("high LH group"), of whom 18 (61%) conceived ($\chi^2=6.15$; $p=0.01$; OR=2.6; 95% CL: 1.2-5.7).

Table 5.1

Pregnancy rate categorised by pre-pregnancy LH concentration

	Pregnant	Not pregnant	Total No.patients
Normal LH	118	29	147
High LH	28	18	46
Total	146	47	193

The distribution of LH concentrations in the 146 women who became pregnant grouped by their pregnancy outcome is shown in Figure 5.2. The vast

majority of women with pre-pregnancy LH concentrations in the normal range (<10iu/l) had a successful pregnancy outcome.

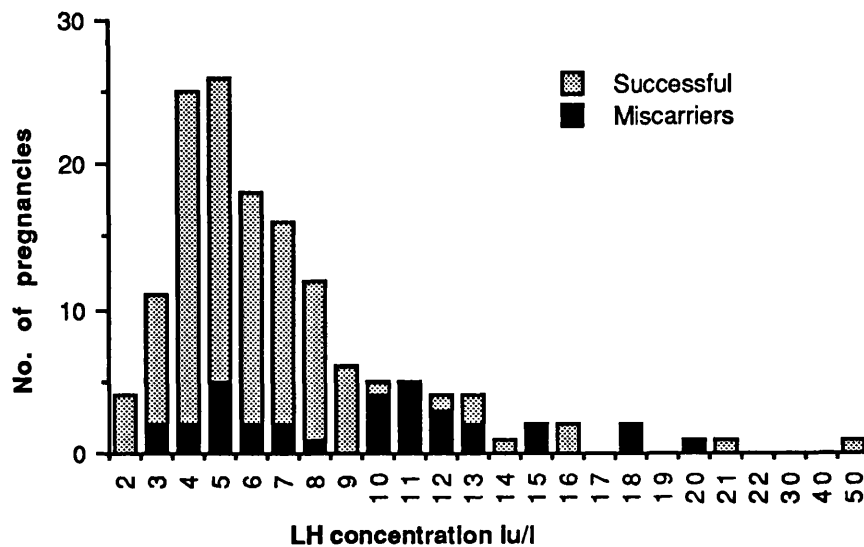


Figure 5.2. Pre-pregnancy LH concentrations in 113 women who had subsequent successful pregnancies and 33 women whose pregnancy ended in miscarriage.

In Table 5.2 the outcome of the 146 pregnancies has been categorised by the prepregnancy LH concentration. In the "normal LH" group 12% ended in a miscarriage, whereas in the high LH group 64% of the 28 pregnancies ended in miscarriage. Nineteen of the total 33 miscarriages (58%) occurred in women who had high LH concentrations, whereas among the 113 successful pregnancies only 8% (9) of the women had had high pre-pregnancy LH concentrations. The difference in the rate of miscarriage between patients in the "high" and "normal" LH groups who became pregnant was highly statistically significant (Fisher's exact probability, $p < 10^{-8}$).

Table 5.2

Pregnancy outcome categorised by pre-pregnancy LH concentration

	Total No. pregnancies	Successful	Miscarriage (%)
Normal LH	118	104	14(12%) ^a
High LH	28	9	19(64%) ^b

a vs. b; $\chi^2=37.4$; $p < 10^{-6}$; OR=15.7; 95% CL: 5.4-46.9.

Analysis of the LH results by the patients' past obstetric history revealed similar results. Of the 26 primigravidae, 20 had had a normal pre-pregnancy LH concentration and 13 of these women have had a successful pregnancy, one woman has had a miscarriage and six women are not yet pregnant. Of the 6 primigravidae with raised LH concentrations, only 2 have had successful pregnancies, 3 have had miscarriages and one has failed to conceive (Table 5.3). The difference in the miscarriage rate was significant (Fisher's exact probability, $p = 0.037$).

Table 5.3.

Pre-pregnancy LH concentrations and subsequent outcome in primigravidae.

	No. patients	Successful pregnancies	Miscarriage (%)
Normal LH	20	13	1(7%) ^a
High LH	6	2	3(60%) ^b

a vs. b; Fishers exact probability, $p=0.037$

There were 17 women who had had no miscarriages, but who gave a history of one or more successful pregnancies ($n=12$) or a previous termination of pregnancy ($n=5$). Within this group 16 (94%) had normal LH concentrations and all of these women went on to have a successful outcome in the pregnancies that occurred during the present study. The one woman with a high LH concentration and past history of a termination of pregnancy and a live child was not pregnant after 12 months.

There were 150 women in the study group whose obstetric history included a miscarriage. One hundred and eleven of them had a normal LH concentration of whom 75 (68%) went on to have a successful pregnancy, 13 (12%) miscarried and 23 (21%) have failed to conceive. In contrast, only 7 (18%) of the 39 women with high LH concentrations had successful pregnancies, 16 (41%) miscarried and 16 (41%) failed to conceive (Fisher's exact probability $p<10^{-6}$).

There were 48 women who had had 1 or 2 miscarriages but no successful pregnancies and 8 of these women did not conceive in the year of this study. Among the 40 pregnancies that did occur, 34 (85%) were to women with a "normal LH", of whom 29 (74%) went on to have successful

pregnancies. Only 2 (33%) of the 6 women in this group with high LH concentrations had successful pregnancies (Fisher's exact probability, $p=0.016$).

A further 30 women gave a history of recurrent abortion, defined as 3 or more consecutive miscarriages (Huisjes 1984) of whom 30% ($n=9$) had high LH concentrations (Table 5.4). Among these 9 women in the "high LH" group, 5 (56%) did not become pregnant during the study period, only one has had a successful pregnancy outcome (11%) and 3 of the 4 women who became pregnant miscarried again (75%). The incidence of miscarriage was significantly lower among the 21 women with a history of recurrent abortions who had normal LH concentrations. Although 8 women have not yet conceived, 12 (57%) of the "normal LH" group have already had a successful pregnancy outcome. The miscarriage rate among the 13 conceptions was only 8% (Fishers' exact probability $p=0.02$)

Table 5.4.

Pre-pregnancy LH concentrations and pregnancy outcome in women with recurrent miscarriage

	Successful	Miscarriage (%)	Total No. patients
Normal LH	12	1(8%) ^a	21
High LH	1	3(75%) ^b	9
Total	13	4(24%)	30

a vs b; Fisher's exact probability, $p=0.02$.

5.4. Discussion.

This prospective study of a population of women intending to become pregnant reports an association of raised serum LH concentrations before pregnancy with infertility and pregnancy loss. Analysing the results both on the basis of past obstetric performance and on subsequent pregnancy outcome, the presence of raised LH concentrations conferred an adverse prognosis. Although the group was heterogenous, with a bias towards women with previous pregnancy losses, the results are of considerable importance, particularly as the LH concentrations were unknown to the subjects or to the investigator.

The women were divided into two groups: those with 'normal' LH concentrations and those with high LH concentrations, the groups being separated at an LH concentration of 10 iu/l. This division was based on data from Eden et al (1988, 1989) and Conway et al (1988) which indicated that LH concentrations in the early follicular phase above this level were outside the normal range. The results from this study demonstrate that follicular LH concentrations in excess of 10iu/l before pregnancy are associated with a poor pregnancy outcome. The question arises as to the cause of the hypersecretion of LH that has been identified. Since the only hypothalamic - pituitary - gonadal disturbance consistently associated with elevated follicular phase LH levels is polycystic ovarian syndrome (PCOS) (Jacobs 1988) it is tempting to suggest that the follicular phase LH measurements in this study have identified women with PCOS. However, it is not possible to conclude from these data whether the poor pregnancy outcome was caused by the exposure of the ovaries to raised hormone concentrations or by an ovarian pathology causing serum LH concentrations to be raised.

Oocytes are normally held in the dictyotene stage of their first meiotic division until just before ovulation. Meiosis has been shown to be inhibited by oocyte maturation inhibitor (OMI), a low molecular weight peptide found in follicular fluid (Winer-Sorgen et al 1986). The production or action of oocyte maturation inhibitor is itself inhibited by the action of LH at mid-cycle (Tsafriri & Pomerantz 1986), thereby ensuring ovulation of an egg matured at the appropriate time. In the sheep, reduced fertilisation of oocytes and abnormal embryo development occurs in vivo after follicle growth in vitro had been carried out in the presence of elevated gonadotrophins (Moor & Trounson 1977).

There is a species specific interval between completion of the first meiotic division of oocytes ("oocyte maturation") and their fertilisation (24-36 hours in man), during which conditions for producing a normal embryo are optimal. Extension of the interval between ovulation and donor insemination (and therefore between the completion of meiosis and fertilisation) is associated with poor rates of fertilisation and a significant increase in the rate of abortion in the rat (Austin 1982) and the human (Guerrero & Rojas 1975)

Jacobs et al (1987) have put forward the hypothesis that when follicular phase LH concentrations are high, as in women with PCOS, that LH penetrates the follicle and permits premature completion of oocyte maturation, resulting in ovulation of an oocyte that is physiologically "aged". Such oocytes would be expected to fertilise poorly and to produce embryos that implant poorly and therefore abort early. This suggestion that inappropriate secretion of LH during follicular development may impair fertilisation and reduce fecundity is supported by the findings in women receiving treatment for subfertility. High serum LH concentrations in the follicular phase of oocyte development are associated with both failure to conceive and a high incidence of pregnancy loss in women who are treated with exogenous gonadotrophin prior to in vitro fertilisation and embryo transfer (Stanger & Yovich 1985, Howles et al 1986, 1987a, Macnamee et al 1987, Punnönen et al 1988) and women with anovulatory infertility receiving LHRH treatment (Eshel et al 1988, Homburg et al 1988). In contrast, women with hypogonadotrophic hypogonadism have a significantly improved pregnancy outcome following treatment with pulsatile LHRH (Abdulwahid et al 1985). The present data extend these observations by demonstrating in a field study an important association of spontaneously elevated LH concentrations and impaired outcome of fertility.

That there is an association between high follicular phase LH concentrations and miscarriage in natural cycles has not been reported previously. These data demonstrate clearly that there is a significant difference in the pre-pregnancy LH concentrations between women who subsequently miscarry their pregnancy and those who go on to have a successful pregnancy ($p < 0.00001$). As a predictive test for pregnancy failure, therefore, an early to mid follicular phase LH concentration appears to be useful. Furthermore these results are complementary to the conclusions obtained in the early pregnancy field study (Chapter 2) in which reproductive history was found to be an important predictive factor in the prognosis for future pregnancy outcome.

For example, based on these endocrinological results, a primigravida with a normal LH concentration has a 93% chance of completing a successful pregnancy, compared with a 40% chance if she has a pre-pregnancy LH concentration greater than 10 iu/l. The incidence of raised LH concentrations in women with a history of live births only was very low (only 6%). Of the women whose obstetric history included live births together with a miscarriage, those with normal LH levels enjoyed a 94% chance of successful pregnancy. Even after one or two miscarriages, a woman with a normal LH can be reassured that she has an 85% chance of having a successful subsequent pregnancy, but if her pre-pregnancy LH level is raised, her chance of successful pregnancy drops to 33%. Among the women with a history of recurrent abortions the same trend was observed. Women with normal LH concentrations had an incidence of successful pregnancy similar to that of primigravidae, but in women with high LH values only 11% had a successful pregnancy.

These correlations suggest that a persistent endocrine abnormality may be the underlying cause of spontaneous abortion in some patients. Although this study did not include ultrasound assessment of ovarian morphology, it is possible that the poor pregnancy outcome noted in the patients with high LH levels was due to women with PCOS. The observations in this study are consistent with the known incidence of PCOS in the general population (Polson et al 1988) and among recurrent aborters (Sagle et al 1988). Whatever the underlying mechanism, a raised serum LH concentration appears to be a significant risk factor for spontaneous abortion. This finding suggests that measurement of follicular phase LH concentrations before pregnancy could become a useful screening test with which to assess a woman's risk of spontaneous abortion. Not only would the finding of an abnormal result be of prognostic value, but in addition could be used to identify patients with an endocrine abnormality which is potentially remediable.

CHAPTER 6.

CONCLUSIONS.

CONCLUSIONS.

These prospective studies have shown that a knowledge of the patient's reproductive history is essential for the clinical assessment of her risk of spontaneous abortion and has provided a quantitative estimate of this risk which can be utilised in clinical practice. Since the risk for primigravidae and women who have had successful pregnancies is low and the most relevant predictive factor for spontaneous abortion is a history of abortion in the previous pregnancy, it would appear that the outcome of a woman's first pregnancy has important consequences for all subsequent pregnancies. The data suggest that women fall into one of two categories of reproducer, those that are successful and those that are not, and contrary to popular belief, that their future reproductive performance can be predicted from the outcome of their first pregnancy. Further studies are needed to identify those factors which determine whether a first pregnancy will result in success or failure. The aim will be to offer prophylactic preconceptual counselling to all nulligravidae.

This view is supported by the observations made in this study about the influence of subfertility on the incidence of abortion. In the Early Pregnancy Loss Field study (Chapter 2), primigravid pregnancies, whether natural or assisted conceptions, shared the same low risk of spontaneous abortion when compared with multigravidae undergoing treatment for subfertility, suggesting that it is the past reproductive history rather than the subfertility treatment that influences the subsequent pregnancy outcome. Furthermore, in the recurrently aborting population reported in Chapter 4, one-third of the patients had a history of a period of relative infertility in the past. This combination of recurrent episodes of abortion together with subfertility had important implications for subsequent pregnancy outcome. This subgroup experienced a significantly higher incidence of abortion in the studied pregnancy when compared with recurrent aborters with no history of subfertility.

The results from the study examining pre-pregnancy luteinising hormone (LH) concentrations provide a possible explanation for these findings. The presence of high LH levels in the follicular phase of the menstrual cycle was strongly correlated with both a past history of spontaneous abortion and pregnancy loss in the studied pregnancy. Furthermore a significant percentage of the patients in the high LH group

failed to conceive during the study period. In the Early Pregnancy Loss Field Study, the incidence of spontaneous abortion in multigravidae who required treatment for subfertility was highest in those patients who had received clomiphene ovulatory induction therapy, and in the study of recurrent aborters, the majority of patients undergoing treatment for subfertility had been given clomiphene. It is tempting to conclude that the recognised dose dependant increase in LH secretion after clomiphene administration was an important factor contributing to these pregnancy losses.

High LH concentrations during the period of oocyte maturation are characteristically found in women with the polycystic ovarian syndrome (PCOS) and recent studies using ultrasound have demonstrated that the incidence of PCOS among recurrent aborters is significantly higher than the prevalence in the general population. Although this study did not include ultrasound assessment of ovarian morphology, it is possible that the poor pregnancy outcome noted in the patients with high LH levels was due to women with PCOS, and has identified the need for a future study combining serum LH monitoring and ultrasonography.

Whatever the underlying mechanism, a raised serum LH concentration appears to be a significant risk factor for spontaneous abortion in the general population as well as amongst a group of recurrent aborters. Not only does this finding offer the possibility of a predictive test for women prior to pregnancy, but suggests that the finding of an abnormal result is remediable. A potential treatment for such patients would be to suppress the high endogenous LH levels with the use of an luteinising hormone releasing hormone (LHRH) analogue, followed by ovulatory induction therapy with exogenous gonadotrophins. It is interesting to note that there was only one abortion among the IVF patients included in the EPL study, and all of these patients had received LHRH analogue treatment prior to their stimulation cycle.

The findings from the EPL Field study demonstrated that women who have one successful pregnancy continue to have successful pregnancies. Only 6% of this group had a further miscarriage. In contrast, women who have one miscarriage have a 20% risk of further miscarriage. It would appear therefore that some factor is present in women with successful pregnancies which protects the fetus against abortion, and conversely that this factor is missing in those women whose pregnancies are repeatedly unsuccessful. However, the results of the study documenting the natural history of anti-paternal cytotoxic

antibody (APCA) were unable to support the hypothesis that the presence of APCA during pregnancy offers protection for a successful pregnancy. Since the development of this antibody is primarily dependent upon the gestational duration of pregnancy, (it is a rare finding in pregnancies of less than 28 weeks gestation), it is unlikely to be relevant to the success or failure of an early pregnancy. Furthermore, detectable APCA disappear from the serum between pregnancies in most women, suggesting that APCA is not a useful serum marker with which to identify patients with recurrent spontaneous abortion.

The results of the screening investigations employed for the patients with recurrent abortion described in Chapter 4 provided an assessment of the usefulness of these tests in the management of these patients. This study concluded that parental karyotyping of both partners, a search for evidence of infection with toxoplasma and documentation of anti-nuclear, anti-cardiolipin, and lupus anticoagulant titres should be performed in all patients. Although the number of patients identified by these tests is small in an unselected population of recurrent aborters, they will identify a group of patients whose pregnancy prognosis could be improved by appropriate treatment, or whose subsequent pregnancy requires prenatal diagnosis and monitoring. The use of hysterosalpingography to identify anatomical abnormalities of the genital tract need only be performed in selected cases where a high index of clinical suspicion is based on the patients' past obstetric history. Evidence of thyroid dysfunction testing and diabetes mellitus were not present in any of the patients studied.

No significant improvement in subsequent pregnancy outcome was observed in the population of recurrent aborters who received paternal white cell immunisation treatment. Seroconversion to APCA positive status had no effect on the pregnancy rate, the interval to conception, nor the outcome of pregnancy. However, patients who became pregnant within 12 weeks of immunisation treatment, irrespective of APCA status, had a significantly lower incidence of spontaneous abortion, lending further weight to the observations discussed earlier that patients with relative subfertility and recurrent abortion have a particularly poor prognosis. It is possible that the finding that one-third of the recurrent aborters had a history of another family member who had experienced several abortions may be a further indication of the importance of PCOS as a causal mechanism in recurrent abortion, since PCOS is known to have a familial distribution.

In conclusion, the results from these prospective studies demonstrate that recurrent spontaneous abortion is a real clinical entity. The incidence of abortion in first pregnancies in the EPL field study was 5%. In women whose first pregnancy succeeded the loss rate in the next pregnancy was 5% also, but those women who had aborted the first pregnancy had a 20% risk of aborting the second. This statistically significant difference shows that the tendency to abort was not random, at least in 20% of those who had aborted the first pregnancy. The incidence of spontaneous abortion in the population of women with a history of multiple pregnancy losses described in Chapter 4, is very significantly higher (34%).

For the individual patient the risk of spontaneous abortion can be predicted from her past reproductive history. Although the factors which determine the outcome of a first pregnancy are at present poorly understood, the results from these studies have identified several areas for further research and have indicated that the assessment of follicular phase LH concentrations could become a useful predictive test which would provide the clinician with a specific and treatable cause of spontaneous abortion.

REFERENCES.

- Abdulwahid NA, Adams J, Van der Spuy ZM, Jacobs HS. Gonadotrophin control of follicular development. *Clin Endocrinol* 1985; 23: 613-626.
- Abraham GE, Maroulis GB, Marshall JR. Evaluation of ovulation and corpus luteum function using measurements of plasma progesterone. *Obstet Gynecol* 1974; 88: 931-933.
- Abramowsky CR, Vegas ME, Swinehart G, Gyves MT. Decidual vasculopathy of the placenta in lupus erythematosus. *N Eng J Med* 1980; 303: 668-672.
- Adamoli A, Bernardi F, Chiaffoni G, Fraccaro M, Giardino D, Romitti L, Simoni G. Reproductive failure and parental chromosome abnormalities. *Hum Reprod* 1986; 1: 99-102.
- Adams J, Franks S, Polson DW, Mason HD, Abdul-Wahid NA, Tucker M, Morris DV, Price J, Jacobs HS. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotrophin releasing hormone. *Lancet* 1985; ii: 1375-1378.
- Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *Br Med J* 1986; 293: 355-359.
- Adashi EY, Rock JA, Sapp KC, Martin EJ, Wentz AC, Jones GES. Gestational outcome of clomiphene related conceptions. *Fertil Steril* 1979; 31: 620-626.
- Adinolfi M. Recurrent habitual abortion, HLA sharing and deliberate immunisation with partner's cells: a controversial topic. *Hum Reprod* 1986; 1: 45-48.
- Adoni A, Palti Z, Milwidsky A, Dolberg M. The incidence of intrauterine adhesions following spontaneous abortion. *Int J Fertil* 1982; 27: 117-118.
- Ager EA, Chin TDY, Poland JD. Epidemic erythema infectiosum. *N Eng J Med* 1966; 275: 1326-1331.
- Ahmed AG, Klopper A. Concomitant secretion of Schwangerschafts protein 1 and human chorionic gonadotrophin following conception. *Clin Endocrinol* 1985; 23: 677-681.
- Ahrons S. HLA antibodies: Influence on the human fetus. *Tissue Antigens* 1971a; 1: 129-136.
- Ahrons S. Leucocyte antibodies: Occurrence in primigravidae. *Tissue Antigens* 1971b; 1: 178-183.
- Aksel S, Jones GES. Effect of progesterone and 17-hydroxyprogesterone caproate on normal corpus luteum function. *Am J Obstet Gynecol* 1974; 118: 466-472.
- Alberman E, Creasy M, Elliott M, Spicer C. Maternal factors associated with fetal chromosomal anomalies in spontaneous abortions. *Br J Obstet Gynaecol* 1976; 83: 621-627.
- Alberman E, Elliott M, Creasy M, Dhadi R. Previous reproductive history in mothers presenting with spontaneous abortions. *Br J Obstet Gynaecol* 1975; 82: 366-373.

- Alberman E, Polani PE, Roberts JAF, Spicer CC, Elliott M, Armstrong E, Dhadiak RK. Parental X-irradiation and chromosome constitution in their spontaneously aborted fetuses. *Ann Hum Genet* 1972; 36: 185-194.
- Alberman E. Maternal age and spontaneous abortion. In: Bennett MJ, Edmonds DK (eds): *Spontaneous and recurrent abortion*. Oxford: Blackwell Scientific Publications, 1987; 77-89.
- Alberman E. The abortus as a predictor of future trisomy 21. In: De la Cruz FF, Gerald PS (eds). *Trisomy 21 (Down syndrome)*. Baltimore: University Park Press, 1981; 69-74.
- Albrechtsen D, Solheim BG, Thorsey E. Antiserum inhibition of the mixed lymphocyte culture (MLC) interaction. Inhibitory effect of antibodies reactive with HLA-D associated determinants. *Cell Immunol* 1977; 28: 258-273.
- Alexander SA, Latinne D, Debruyere M, Dupont E, Gottlieb W, Thomas K. Belgian experience with repeat immunisation in recurrent spontaneous abortions. In: Beard RW, Sharp F (Eds): *Early pregnancy loss: Mechanisms and treatment*. Springer-Verlag, 1988: 355-363.
- Alkalay AL, Pomerance JJ, Rinion DL. Fetal varicella syndrome. *J Pediatr* 1987; 111: 320-323.
- Allen WM, Corner GW. Physiology of corpus luteum; normal growth and implantation of embryos after very early ablation of ovaries and influence of extracts of corpus luteum. *Am J Physiol* 1929; 88: 340-346.
- Altshuler G. Immunologic competence of the immature human fetus. Morphologic evidence from intrauterine cytomegalovirus infection. *Obstet Gynecol* 1974; 43: 811-816.
- Anand A, Gray ES, Brown T, Clewley JP, Cohen BJ. Human parvovirus infection in pregnancy and hydrops fetalis. *N Eng J Med* 1987; 316: 183-186.
- Andrews MC, Mausher SJ, Levy DL, Jones HW, Garcia JE, Rosenwaks Z, Jones GS, Acosta AA. An analysis of the obstetric outcome of 125 consecutive pregnancies conceived in vitro and resulting in 100 deliveries. *Am J Obstet Gynecol* 1986; 154: 848-854.
- Andrews T, Roberts DF. Chromosome analyses in couples with repeated pregnancy loss. *J Biosoc Sci* 1982; 14: 33-52.
- Andrews WC. Luteal phase defects. *Fertil Steril* 1979; 32: 501-509.
- Angell RR, Aitken RJ, Van Look PFA, Lumsden MA, Templeton AA. Chromosome abnormalities in human embryos after in vitro fertilisation. *Nature* 1983; 303: 336-338.
- Angell RR, Templeton AA, Aitken RJ. Chromosome studies in human in vitro fertilisation. *Hum Genet* 1986; 72: 333-339.
- Anthony GS, Calder AA, MacNaughton MC. Cervical resistance in patients with previous spontaneous midtrimester abortion. *Br J Obstet Gynaecol* 1982; 89: 1046-1049.
- Armar NA, Adams J, Jacobs HS. Induction of ovulation with gonadotrophin releasing hormone. In: Bonnar J (ed): *Recent Advances in Obstetrics and Gynaecology*. London, Churchill Livingstone 1986; 15: 259-277.

- Asch RH, Greenblatt RB. Update on the safety and efficacy of clomiphene citrate as a therapeutic agent. *J Reprod Med* 1976; 17: 175-180.
- Asherman JG. Amenorrhoea traumatica (atretica). *J Obstet Gynaecol Br Commonw* 1948; 55: 23-30.
- Asherman JG. Traumatic intrauterine adhesions. *J Obstet Gynaecol Br Commonw* 1950; 57: 892-896.
- Askrog V, Harvald B. Teratogenic effect of anaesthetic inhalations. *Nord Med* 1970; 83: 498-504.
- Austin CR. The egg. In: Austin CR, Short RE (Eds): *Reproduction in mammals. Part 1. Germ cells and fertilisation*. 2nd edition. Cambridge: Cambridge University Press, 1982, 58-73.
- Awan AK. Some biologic correlates of pregnancy wastage. *Am J Obstet Gynecol* 1974; 119: 525-531.
- Axellson G, Rylander R. Exposure to anaesthetic gases and spontaneous abortion. Response bias in a postal questionnaire study. *Int J Epidemiol* 1982; 11: 250-256.
- Babaknia A, Rock JA, Jones HWJr. Pregnancy success following abdominal myomectomy for infertility. *Fertil Steril* 1978; 30: 644-647.
- Baird DT, Baker TG, McNatty KP, Neal P. Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle. *J Reprod Fertil* 1975; 45: 611-619.
- Bakketeig LS, Hoffman HJ. Perinatal mortality by birth order within cohorts based on sibship size. *Br Med J* 1979; 2: 693-696.
- Balasch J, Creus M, Márquez M, Burzaco I, Vanrell JA. The significance of luteal phase deficiency on fertility: a diagnostic and therapeutic approach. *Hum Reprod* 1986; 1: 145-147.
- Balasch J, Ercilla G, Vanrell JA, Vives J, Gonzales-Merlo J. Effects of HLA antibodies on pregnancy. *Obstet Gynaecol* 1981; 57: 444-446.
- Barnes AB, Colton T, Gunderson J, Noller KL, Tilley BC, Strama T, Townsend DE, Hatab P, O'Brien PC. Fertility and outcome of pregnancy in women exposed in utero to diethylstilboestrol. *N Eng J Med* 1980; 302: 609-613.
- Barnstable CJ, Bodmer WF. Immunology and the fetus. *Lancet* 1978; i: 326.
- Baron JA. Smoking and estrogen-related disease. *Am J Epidemiol* 1984; 119: 9-22.
- Bartizal FJ, Pacheco JC, Malkasian GD, Washington JA. Microbial flora found in the products of conception in spontaneous abortion. *Obstet Gynecol* 1974; 43: 109-112.
- Baulieu E-E. RU-486: An antiprogesterone steroid with contragestive activity in women. In: Baulieu E-E, Segal SJ (eds): *The antiprogesterone steroid RU-486 and human fertility control*. New York: Plenum Press, 1985; 1-26.
- Beard RW, Mowbray JF, Pinker GD. Miscarriage or abortion? *Lancet* 1985; ii: 1123.
- Beer AE, Billingham RE. Host responses to intrauterine tissue, cellular and fetal allografts. *J Reprod Fertil* 1974a; 21: 59-88.
- Beer AE, Billingham RE. The embryo as a transplant. *Sci Am* 1974b; 230: 36-40.

- Beer AE, Quebbeman JF, Semprini AE, Smouse PE, Haines RF. Recurrent abortions: Analysis of the roles of parental sharing of histocompatibility antigens and maternal immunological responses to paternal antigens. In: Isojima S, Billington WE (eds): *Reproduction and Immunology*. Amsterdam, Elsevier 1983; 185-195.
- Beer AE, Quebbeman JF, Ayers JT, Haines RF. Major histocompatibility complex antigens, maternal immune responses and chronic habitual abortion in humans. *Am J Obstet Gynaecol* 1981; 141: 987-999.
- Beer AE, Semprini AE, Ziaoyu Z, Quebbeman JF. Pregnancy outcome in human couples with recurrent spontaneous abortions: HLA antigen profiles; HLA antigen sharing; Female serum MLR blocking factors; and paternal leucocyte immunisation. *Exp Clin Immunogenet* 1985; 2: 137-153.
- Beer AE, Sio JO. Placenta as an immunological barrier. *Biol Reprod* 1982; 26: 15-27.
- Beer AE. Immunopathologic factors contributing to recurrent spontaneous abortion in humans. *Am J Reprod Immunol* 1983; 4: 182-184.
- Beer AE. Pregnancy outcome in couples with recurrent abortions following immunological evaluation and therapy. In: Beard RW, Sharp F (eds): *Early pregnancy loss: Mechanisms and treatment*. Springer-Verlag, 1988: 337-349.
- Behrman SJ. Artificial insemination. *Clin Obstet Gynaecol* 1979; 22: 245-253.
- Bell JE, Gosden CM. Central nervous system abnormalities - contrasting patterns in early and late pregnancy. *Clin Genet* 1978; 13: 387-396.
- Ben-Baruch G, Menczer J, Mashiach S, Serr DM. Uterine anomalies in diethylstilboestrol-exposed women with fertility disorders. *Acta Obstet Gynecol Scand* 1981; 60: 395-397.
- Ben-Rafael Z, Fateh M, Flickinger GL, Tureck R, Blasco L, Mastroianni L. Incidence of abortion in pregnancies after in vitro fertilisation and embryo transfer. *Obstet Gynaecol* 1988; 71: 297-300.
- Bender S. The end-results in primary sterility. *Br Med J* 1952; 2: 409-413.
- Bennett MJ. Congenital abnormalities of the fundus. In: Bennett MJ, Edmonds DK (eds). *Spontaneous and recurrent abortion*. Oxford: Blackwell Scientific Publications, 1987; 109-129.
- Berah M, Jacobowicz, Graydon JJ. Leuco-agglutinins in normal and abnormal pregnancy. *Med J Aust* 1966; 53: 1030-1033.
- Berger MJ, Goldstein DP. Impaired reproductive performance in DES exposed women. *Obstet Gynecol* 1980; 55: 25-27.
- Bergquist CA, Rock JA, Jones HWJr. Pregnancy outcome following treatment of intrauterine adhesions. *Int J Fertil* 1981; 26: 107-111.
- Berman SM, Harrison HR, Boyce WT. Low birth weight, prematurity and postpartum endometritis. *JAMA* 1987; 257:1189-1194.
- Berry CL. The examination of embryonic and fetal material in diagnostic histopathology laboratories. *J Clin Pathol* 1980; 33: 317-326.
- Biddle PK, Friedman CI, Johnson PM. Lymphocyte-reactive antibodies and recurrent early pregnancy failure. *Am J Obstet Gynecol* 1987; 157: 785-786.

- Billewicz WZ. Some implications of self selection for pregnancy. *Br J Prev Soc Med* 1973; 27: 49-52.
- Billington WD. Influence of immunological disparity of mother and fetus on size of placenta in mice. *Nature* 1964; 202: 317-318.
- Binkin NJ. Trends in induced legal abortion morbidity and mortality. *Clin Obstet Gynaecol* 1986; 13: 83-93.
- Birkeland SA, Kristoffersen K. The fetus as an allograft: a longitudinal study of normal human pregnancies studied with mixed lymphocyte cultures between mother-father and mother-child. *Scand J Immunol* 1980; 11: 311-319.
- Bischof P. Pregnancy-associated plasma protein-A: an inhibitor of the complement system. *Placenta* 1981; 2: 29-34.
- Bishop PMF. Habitual abortion, its incidence and treatment with progesterone or vitamin E. *Guy's Hospital Report* 1937; 17: 362-371.
- Blackwell R, Chang A. Video display terminals and pregnancy. A review. *Br J Obstet Gynaecol* 1988; 95: 446-453.
- Bloom AD. Guidelines for studies of human populations exposed to mutagenic and reproductive hazards. *Proc Birth Defects Foundation, Washington DC* 1981; 37.
- Blumberg BD, Shulkin JD, Rotter JI, Mohandas T, Kaback MM. Minor chromosome variants and major chromosomal anomalies in couples with recurrent abortion. *Am J Hum Genet* 1982; 34: 948-960.
- Bohnet HG, Dato K, Trapp M, Weise HC, Leidenberger FA. Different hormonal patterns in human menopausal gonadotrophin-treated, clomiphene citrate-treated and untreated conception cycles. *Fertil Steril* 1986; 45: 469-474.
- Booth CM, Booth AG. Immunoelectrophoretic evidence that the human trophoblast transferrin receptor is identical to a major plasma membrane antigen present throughout pregnancy. *Placenta* 1982; 3: 57-66.
- Botella-Lhuissia J. The endometrium in repeated abortion. *Int J Fertil* 1962; 7: 147-154.
- Boué A, Boué J, Gropp A. Cytogenetics of pregnancy wastage. *Adv Hum Genet* 1985; 14: 1-57.
- Boué A, Boué J. Le rôle des anomalies chromosomiques dans les échecs de la reproduction. *J Gynécol Obstet Biol Reprod* 1977; 6: 5-21.
- Boué A, Gallano P. A collaborative study of the segregation of inherited structural arrangements in 1356 prenatal diagnoses. *Prenat Diagn* 1984; 4: 45-67.
- Boué J, Boué A, Lazar P. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. *Teratology* 1975; 12: 11-26.
- Boué J, Boué A. Anomalies chromosomiques dans les avortements spontanés. In: Boué A & Thibault C. Chromosomal errors in relation to reproductive failure. INSERM Paris 1973b.

- Boué J, Boué A, Lazar P. The epidemiology of human spontaneous abortions with chromosomal anomalies. In: Blandau RJ (ed) *Aging gametes*. Basel: Karger, 1975a; 330-348.
- Boué J, Boué A. Chromosomal analysis of two consecutive abortuses in each of 43 women. *Humangenetik* 1973a; 19: 275-280.
- Boué J, Phillippe E, Giroud A, Boué A. Phenotypic expression of lethal chromosomal anomalies in human abortuses. *Teratology* 1976; 14: 3-20.
- Boué JG, Boué A, Lazar P, Gueguen S. Outcome of pregnancies following a spontaneous abortion with chromosomal anomalies. *Am J Obstet Gynecol* 1973; 116: 806-842.
- Boué JG, Boué A. Increased frequency of chromosomal anomalies in abortions after induced ovulation. *Lancet* 1973c; i: 679-680.
- Brambati B, Lanzani A, Oldrini A. Transabdominal chorionic villus sampling: clinical experience of 1159 cases. *Prenat Diagn* 1988; 8: 609-617.
- Branch DW, Scott JR, Kochenour NK, Hershegold E. Obstetric complications associated with the lupus anticoagulant. *N Eng J Med* 1985; 313: 1322-1326.
- Brazin SA, Simkovich JW, Johnson WT. Herpes zoster during pregnancy. *Obstet Gynecol* 1979; 53: 175-181.
- Bresnihan B, Oliver M, Grigor RR, Lewkonja RM, Hughes GR, Lovins RE, Faulk WP. Immunological mechanism for spontaneous abortion in systemic lupus erythematosus. *Lancet* 1977; ii: 1205-1207.
- Brodsky JB, Cohen EN, Brown BW, Wu ML, Whitcher C. Surgery during pregnancy and fetal outcome. *Am J Obstet Gynecol* 1980; 138: 1165-1167.
- Brook I, Feingold M, Schwartz A, Zakut H. Ultrasonography in the diagnosis of cervical incompetence in pregnancy- a new diagnostic approach. *Br J Obstet Gynaecol* 1981; 88: 640-643.
- Brosens I, Robertson WB, Dixon H G. The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstet Gynecol Ann* 1972; 1: 177-191.
- Brotherton J, Craft IL. A clinical and pathological survey of 91 cases of spontaneous abortion. *Fert Steril* 1972; 23: 289-294.
- Brown KE. What threat is human parvovirus B19 to the fetus? *Br J Obstet Gynaecol* 1989; 96: 764-767.
- Bulmer JN, Billington WD, Johnson PM. Immuno-histologic identification of trophoblast populations in early human pregnancy with the use of monoclonal antibodies. *Am J Obstet Gynecol* 1984; 148: 19-26.
- Bulmer JN, Johnson PM, Bulmer D. Leucocyte populations in human decidua and endometrium. In: Gill TJ, Wegmann TG, Nisbet-Brown E (eds): *Immunoregulation and fetal survival*. New York, Oxford University Press; 1987, 111-134.
- Bulmer JN, Sunderland CA. Immunohistological characterisation of lymphoid cell populations in the early human placental bed. *Immunol* 1984; 52: 349-357.
- Burke J, Johannsen K. The formation of HLA antibodies in pregnancy. The antigenicity of aborted and term fetuses. *J Obstet Gynaecol Br Commw* 1974; 81: 222-228.

- Buttram VCJr, Gibbons WE. Müllerian anomalies: a proposed classification (an analysis of 144 cases). *Fertil Steril* 1979; 32: 40-46.
- Buttram VCJr, Reiter RC. Uterine leiomyomata: etiology, symptomatology and management. *Fertil Steril* 1981; 36: 433-445.
- Buttram VCJr, Turati G. Uterine synechiae: variations in severity and some conditions which may be conducive to severe adhesions. *Int J Fertil* 1977; 22: 98-104.
- Byrd JF, Askew DE, McDonough PG. Cytogenetic findings in fifty-five couples with recurrent fetal wastage. *Fertil Steril* 1977; 28: 246-250.
- Campana M, Serra A, Neri G. Role of chromosome aberrations in recurrent abortion: A study of 269 balanced translocations. *Am J Med Genet* 1986; 24: 341-356.
- Canadian collaborative CVS-Amniocentesis Clinical Trial Group. First report: Multicentre Randomised Clinical Trial of Chorion Villus Sampling and Amniocentesis. *Lancet* 1989; i: 2-6.
- Cannon DSH. Malaria and prematurity in the Western region of Nigeria. *Br Med J* 1958; 2: 877-878.
- Carp HJA, Toder V, Gazit E, Orgad S, Mashiach S, Serr DM, Nebel L. Paternal leucocyte immunisation and habitual abortion. *Contemp Rev Obstet Gynaecol* 1988; 1: 49-59.
- Carr DH. Chromosome anomalies as a cause of spontaneous abortion. *Am J Obstet Gynecol* 1967; 97: 283-293.
- Carreras LO, Defreyn G, Machin SJ, Vermynen J, Van Asche A, Renaer M. Arterial thrombosis, intrauterine death and the "lupus" anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. *Lancet* 1981a; i: 244-246.
- Carreras LO, Vermynen J, Spitz B Van Asche A. Lupus anticoagulant and inhibition of prostacyclin formation in patients with repeated abortion, intrauterine growth retardation and intrauterine death. *Br J Obstet Gynaecol* 1981b; 88: 890-894.
- Carrington D, Gilmore DH, Whittle MJ, Aitken D, Gibson AA, Patrick WJ, Brown T, Caul EO, Field AM, Clewley JP, Cohen BJ. Maternal serum alpha-fetoprotein- a marker of fetal aplastic crisis during intrauterine human parvovirus infection. *Lancet* 1987; i: 433-435.
- Carter J, Sargent IL, Redman CWG. Antibodies in human pregnancy. *J Reprod Immunol Suppl* 1986; 105.
- Casagrande JT, Pike MC, Henderson BE. Re: Menarcheal age and spontaneous abortion. *Am J Epidemiol* 1982; 115: 481-483.
- Cashner KA, Christopher CR, Dysert GA. Spontaneous fetal loss after demonstration of a live fetus in the first trimester. *Obstet Gynecol* 1987; 70: 827-830.
- Caspersson T, Zech L, Johansson C, Modest EJ. Identification of human chromosomes by DNA-binding fluorescent agents. *Chromosoma* (Berlin) 1970; 30: 215-227..
- Caudle MR, Rote NS, Scott JR, DeWitt C, Barney MF. Histocompatibility in couples with recurrent spontaneous abortion and normal fertility. *Fertil Steril* 1983; 39: 793-798.

- Ceppellini R, Bonnard GD, Coppo F. Mixed lymphocyte cultures and HLA antigens:1. Reactivity of young fetuses, newborns and mothers at delivery. *Transpl Proc* 1971; 3: 58-63.
- Cerni C, Tatra G, Bohn H. Immunosuppression by human placental lactogen and pregnancy-specific β 1-glycoprotein. *Arch Gynaek* 1977; 223: 1-7.
- Chalmers JA. Endometriosis. London, Butterworths 1975.
- Chan JK, Harris EN, Hughes GRV. Successful pregnancy following suppression of anti-cardiolipin antibody and lupus anticoagulant with azathioprine in systemic lupus erythematosus. *J Obstet Gynaecol* 1986; 7: 16-17.
- Chandley AC. Cytogenetics and infertility in man. *Ann Hum Genet* 1975; 39: 231-254.
- Chandley AC. The origin of chromosomal aberrations in man and their potential for survival and reproduction in the adult human population. *Ann Hum Genet* 1981; 24: 5-11.
- Chartier M, Roger M, Barrat J, Michelon B. Measurement of plasma human chorionic gonadotrophin (hCG) and beta-hCG activities in the late luteal phase: evidence of the occurrence of spontaneous menstrual abortion in infertile women. *Fertil Steril* 1979; 31: 134-137.
- Chen C, Jones WR, Bastin F, Forde C. Monitoring embryos after in vitro fertilisation using early pregnancy factor. *Ann New York Acad Sci* 1985; 442: 420-428.
- Chez RA. Proceedings of the symposium 'Progesterone, progestins and fetal development'. *Fertil Steril* 1978; 30: 16-26.
- Christianens GCML, Stoutenbeek P. Spontaneous abortion in proven intact pregnancies. *Lancet* 1984; ii: 571-572.
- Christiansen OB, Grunnet N, Anderson ES, Lauritsen JG. Blood transfusions in habitual abortion. *Fertil Steril* 1988; 49: 186.
- Christiansen OB, Riisom K, Lauritsen JG. No increased histocompatibility antigen-sharing in couples with idiopathic habitual abortions. *Hum Reprod* 1989; 4: 160-162.
- Chung CS, Smith RG, Steinhoff PG, Ming-Pi M. Induced abortion and spontaneous fetal loss in subsequent pregnancies. *Am J Publ Hlth* 1982; 72: 548-554.
- Clark DA, Mowbray JF, Underwood JL, Liddell H. Histopathologic alterations in the decidua in human spontaneous abortion: loss of cells with large cytoplasmic granules. *Am J Reprod Immunol Microbiol* 1987; 13: 19-22.
- Clarke B, Kirby DRS. Maintenance of histocompatibility polymorphisms. *Nature* 1974; 211: 999-1000.
- Clarke CA, Hobson D, McKendrick OM, Rogers SC, Sheppard PM. Spina bifida and anencephaly: miscarriage as possible cause. *Br Med J* 1975; 4: 743-746.
- Clarke DA. Host immunoregulatory mechanisms and the success of the conceptus fertilised in vivo and in vitro. In: Beard RW, Sharp F (eds): Early pregnancy loss: Mechanisms and treatment. Springer-Verlag, 1988; 215-228.

- Clarren SK, Smith DW. The fetal alcohol syndrome. *N Eng J Med* 1978; 298: 1063-1067.
- Cline DL. Unsuspected subclinical pregnancies in patients with luteal phase defects. *Am J Obstet Gynecol* 1979; 134: 438-444.
- Cocchiara R, DiTrapani G, Azzolina A, Cittadini E, Geraci D. Binding of human EPF to receptors on PBMC as a first signal of pregnancy immuno-modulation. *Hum Reprod* 1986; 1: 33-36.
- Cohen BH. ABO and Rh incompatibility. i. Fetal and neonatal mortality with ABO and Rh incompatibility: some new interpretations. *Am J Hum Genet* 1970; 22: 412-433.
- Cohen EN, Bellville JW, Brown BW. Anaesthesia, pregnancy and miscarriage. A study of operating room nurses and anaesthetists. *Anaesthes* 1971; 35: 343-347.
- Cohen EN, Brown BW, Wu ML, Whitcher CE. Occupational disease in dentistry and chronic exposure to trace anaesthetic gases. *J Am Dent Ass* 1980; 101: 21-28.
- Conley CL, Hartmann RC. A haemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J Clin Invest* 1952; 31: 621-622.
- Contractor SF, Davies H. Effect of human chorionic gonadotrophin on phytohaemagglutinin induced lymphocyte transformation. *Nature (New Biology)* 1973; 243: 284-286.
- Conway GS, Honour JW, Jacobs HS. Heterogeneity of the polycystic ovary syndrome: Clinical, endocrine and ultrasound features in 556 patients. *Clin Endocrinol* 1989; 30: 459-470.
- Cooke ID, Morgan CA, Parry TE. Correlation of endometrial biopsy and plasma progesterone levels in infertile women. *J Obstet Gynaecol Br Commonw* 1972; 79: 647-650.
- Correy JF, Marsden DE, Schokman FCM. The outcome of pregnancy resulting from clomiphene-induced ovulation. *Aust NZ J Obstet Gynaecol* 1982; 22: 18-21.
- Corson SL. Factors affecting donor artificial insemination success rates. *Fertil Steril* 1980; 33: 415-422.
- Coulam CB, Moore SB, O'Fallon WM. Association between major histocompatibility antigens and reproductive performance. *Am J Reprod Immunol Microbiol* 1987; 14: 54-58.
- Cousins L, Karp W, Lacey C, Lucas WE. Reproductive outcome of women exposed to DES in utero. *Obstet Gynecol* 1980; 56: 70-74.
- Couzinet B, Lestart N, Brailly S, Forest M, Schiason G. Stimulation of ovarian follicular maturation with follicle stimulating hormone in women with gonadotrophin deficiency. *J Clin Endocrinol Metab* 1988; 66: 552-556.
- Covell G. Congenital malaria. *Trop Dis Bull* 1950; 47: 1147-1167.
- Covone A, Mutton ED, Johnson PM, Adinolfi M. Trophoblast cells in peripheral blood from pregnant women. *Lancet* 1984; ii: 841-843.

- Cowchock S, Dehoratius RD, Wapner RJ, Jackson LG. Subclinical autoimmune disease and unexplained abortion. *Am J Obstet Gynecol* 1984; 150: 367-371.
- Cowchock S, Smith JB, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. *Am J Obstet Gynecol* 1985; 155: 1002-1010.
- Craig CJT. Congenital abnormalities of the uterus and foetal wastage. *Obstet Gynecol Surv* 1974; 29: 612-614.
- Cramp HE, Armstrong BDJ. Erythema infectiosum: no evidence of teratogenicity. *Br Med J* 1977; 1: 1031.
- Crandall BF, Howard J, Lebherz TB, Rubinstein L, Sample W, Sarti D. Follow up of 2000 second trimester amniocenteses. *Obstet Gynecol* 1980; 56:625-628.
- Crane JP, Chung SW. An embryonic model to explain cytogenetic inconsistencies observed in chorionic villus versus fetal tissue. *Prenat Diagn* 1988; 8: 119-129.
- Crane JP, Wahl N. The role of maternal diabetes in repetitive spontaneous abortion. *Fertil Steril* 1981; 36: 477-479.
- Creasy MR, Crolla JA, Alberman E. A cytogenetic study of human spontaneous abortions using banding techniques. *Hum Genet* 1976; 31: 177-196.
- Creasy R. The cytogenetics of spontaneous abortion in humans. In: Beard RW, Sharp F (eds): *Early pregnancy loss: Mechanisms and treatment*. Springer-Verlag, 1988; 293-304.
- Crombleholme WR, Minkoff HL, Delke I, Schwartz RH. Cervical cerclage: an aggressive approach to threatened or recurrent pregnancy wastage. *Am J Obstet Gynecol* 1983; 146: 168-174.
- Csapo AI, Pulkinnen MO, Ruttner B, Sauvage JP, Wiest WG. The significance of the human corpus luteum in pregnancy maintenance. *Am J Obstet Gynecol* 1972; 112: 1061-1067.
- Csapo AI, Pulkinnen MO, Wiest WG. Effects of lutectomy and progesterone replacement therapy in early pregnant patients. *Am J Obstet Gynecol* 1973; 115: 759-765.
- Cure S, Boué J, Boué A. Growth characteristics of human cell lines with chromosomal anomalies. *Biomedicine (Express)* 1974; 21: 233-236.
- Daffos F, Forestier F, Capella-Pavlovsky M, Thulliez P, Aufrant C, Valenti D, Cox WL. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. *N Eng J Med* 1988; 318: 271-275.
- Daling JR, Emmanuel I. Induced abortion and subsequent pregnancy outcome in a series of American women. *N Eng J Med* 1977; 297: 1241-1245.
- Daly DC, Walters CA, Riddick DH, Soto-Albors CE. Hysteroscopic metroplasty: surgical technique and obstetric outcome. *Fertil Steril* 1983; 39: 623-628.
- Davies DP, Gray OP, Ellwood PC, Abernethy M. Cigarette smoking in pregnancy: associations with maternal weight gain and fetal growth. *Lancet* 1976; i: 385-387.
- Davies M, Brown CM. Antitrophoblast antibody responses during normal human pregnancy. *J Reprod Immunol* 1985; 7: 285-297.

- Davies M. An ELISA for the detection of maternal antitrophoblast antibodies in human pregnancy. *J Immunol Meth* 1985; 77: 109-118.
- Davies M. Antigenic analysis of immune complexes formed in normal human pregnancy. *Clin Exp Immunol* 1985; 61: 406-415.
- Davis JR, Weinstein L, Veomett IC, Shenker L, Giles HR, Hauck L. Balanced translocation karyotypes in patients with repetitive abortion. *Am J Obstet Gynecol* 1982; 144: 229-233.
- Daya S, Clark DA, Devlin C, Jarrell J, Chaput A. Suppressor cells in human decidua. *Am J Obstet Gynecol* 1985a; 151: 267-270.
- Daya S, Clark DA, Devlin C, Jarrell J. Preliminary characterisation of two types of suppressor cells in the human uterus. *Fertil Steril* 1985b; 44: 778-785.
- Daya S, Clark DA. Immunosuppressive factor produced by human embryos in vitro. *N Engl J Med* 1986; 315: 1551-1552.
- Daya S, Lee S, Underwood JL, Mowbray JF, Craft IL, Clark DA. Prediction of outcome following transfer of in vivo fertilised human embryos by measurement of embryo associated suppressor factor. In: Clark DA, Croy BA (eds): *Reproductive Immunology*. Amsterdam: Elsevier 1986; 277-285.
- DeCherney A, Polan ML. Hysteroscopic management of intrauterine lesions and intractable uterine bleeding. *Obstet Gynecol* 1983; 61: 392-397.
- Dekaban A, Baird R. Fetal wastage, mortality, and morbidity in the offspring of diabetic and normal control mothers. *J Pediatr* 1959; 55: 563-576.
- Deligdish L. Endometrial changes associated with myomatous uterus. *J Clin Pathol* 1970; 23: 676-681.
- Denning J V. Visual display units - Biomedical aspects of their use. London School of Hygiene and Tropical Medicine, London 1985.
- Derksen RHW, Biesma D, Bouma B, Gmelig Meyling FH, Kater L. Discordant effects of prednisone on anti-cardiolipin antibodies and the lupus anticoagulant. *Arthritis Rheum* 1986; 29: 1295-1296.
- Derue GJ, Englert HJ, Harris EN, Gharavi AE, Morgan SH, Hull RG, Elder MG, Hawkins DF, Hughes GRV. Fetal loss in systemic lupus erythematosus: association with anti-cardiolipin antibodies. *J Obstet Gynaecol* 1985; 5: 207-209.
- Desdepova TS, Manev KH, Ianakieva M. The role of listeriosis in the etiology of spontaneous habitual abortion. *Akush Ginekol (Sofia)* 1978; 1: 396-402.
- Desmonts G, Couvreur J. Congenital toxoplasmosis: a prospective study of 378 pregnancies. *N Eng J Med* 1974; 290: 1110-1116.
- Desmonts G, Daffos F, Forestier F, Capella-Pavlovsky M, Thulliez P, Chartier M. Prenatal diagnosis of congenital toxoplasmosis. *Lancet* 1985; i: 500-504.
- DeWolf F, Carreras LO, Moerman P V, Vermynen J, Van Asche A, Renaer M. Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss and a lupus anticoagulant. *Am J Obstet Gynecol* 1982; 142: 829-834.
- Dieckmann WJ, Davis ME, Rynkiewicz LM, Pottinger RE. Does the administration of diethylstilboestrol during pregnancy have therapeutic value? *Am J Obstet Gynecol* 1953; 66: 1062-1081.

- DiMusto JC, Bohjalian O, Millar M. Mycoplasma hominis type 1 infection and pregnancy. *Obstet Gynecol* 1973; 41: 33-37.
- DiZerega GS, Hodgen GD. Luteal phase dysfunction infertility: a sequel to aberrant folliculogenesis. *Fertil Steril* 1981; 35: 489-499.
- Dmowski WP, Greenblatt RB. Asherman's syndrome and risk of placenta accreta. *Obstet Gynecol* 1968; 34: 288-299.
- Doughty RW, Gelsthorpe K. A scheme for screening blood samples to acquire lymphocyte cytotoxic antisera for tissue typing and results of an initial screening programme. *Transplantation* 1971; 12: 429-433.
- Downs KA, Gibson M. Basal body temperature graph and the luteal phase defect. *Fertil Steril* 1983; 40: 466-468.
- Dreskon RB, Spicer SS, Greene WB. Ultrastructural localisation of chorionic gonadotrophin in human term placentae. *J Histochem Cytochem* 1970; 18: 862-874.
- Driscoll SG, Kundsins RB, Horne HWJr, Scott JM. Infections and first trimester losses: Possible role of mycoplasmas. *Fertil Steril* 1969; 20: 1017-1019.
- Drury MI, Greene AT, Stronge JM. A study of 600 pregnancies. Pregnancy complicated by clinical diabetes mellitus. *J Obstet Gynecol* 1977; 49: 519-523.
- Dyson JL, Kohler HG. Anencephaly and ovulation stimulation. *Lancet* 1973; i: 1256-1257.
- Eastman NJ. Habitual abortion. In: Meigs JV, Sturgis S (eds): *Progress in Obstetrics and Gynaecology*. Vol 1. New York, Grune & Stratton 1946; 262-266.
- Eden JA, Place J, Carter GD, Jones J, Alaghband-Zadeh J, Pawson M. The diagnosis of polycystic ovaries in subfertile women. *Br J Obstet Gynaecol* 1989; 96: 809-815.
- Eden JA, Place J, Carter GD, Alaghband-Zadeh J, Pawson M. Is the polycystic ovary a cause of infertility in the ovulatory woman? *Clin Endocrinol* 1989; 30: 77-82.
- Editorial BMJ. Brucellosis. *Br Med J* 1981; 282: 1180.
- Editorial Lancet. Alcohol and spontaneous abortion. *Lancet* 1980; ii: 188.
- Editorial Lancet. Perinatal listeriosis. *Lancet* 1980; i: 911.
- Edmonds DK, Lindsay KS, Miller JF, Williamson E, Wood PJ. Early embryonic mortality in women. *Fertil Steril* 1982; 38: 447-453.
- Edwards RG, Fishel SB, Cohen J, Fehilly CB, Purdy JM, Slater JM, Steptoe PC, Webster JM. Factors influencing the success of In Vitro Fertilisation and Embryo Transfer. *J In Vitro Fert Embryo Transfer* 1984; 1: 3-23.
- Edwards RG, Steptoe PC. Current status of in-vitro fertilisation and implantation of human embryos. *Lancet* 1983; ii: 1265-1269.
- Eisinger SH. Second-trimester spontaneous abortion, the IUD, and infection. *Am J Obstet Gynecol* 1976; 124: 393-397.
- Elbel H, Prokop O, Schleyer F. Der Lewis-blutfaktor als ursache von reihenfehlgeburten. *Geburtsh Frauenheik* 1952; 12: 204-206.
- Elias M, Eldor A. Thromboembolism in patients with the 'lupus'-type circulating anticoagulant. *Arch Intern Med* 1984; 144: 510-515.

- Ellis SA, Sargent IL, Redman CWG, McMichael AJ. Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line. *Immunol* 1986; 59: 595-601.
- Elsacker-Niele AM, Salimans MM, Weiland HT, Vermey-Keers C, Andersom MJ, Versteeg J. Fetal pathology in human parvovirus B19 infection. *Br J Obstet Gynaecol* 1989; 96: 768-775.
- Engelfriet CP, Britten A. The cytotoxic test for leucocyte antibodies. *Vox Sang* 1965; 10: 660-674.
- Erhardt CL. Pregnancy losses in New York City, 1960. *Am J Publ Health* 1963; 53: 1337-1352.
- Erikson A, Källén B. Hospitalisation for miscarriages and delivery outcome among Swedish nurses working in operating rooms. 1973-1978 *Anesth Analg* 1985; 64: 981-988.
- Eshel A, Abdulwahid NA, Armar NA, Adams J, Jacobs HS. Pulsatile luteinising hormone releasing hormone therapy in women with polycystic ovary syndrome. *Fertil Steril* 1988; 49: 956-960.
- Evans DR. Neural tube defects: importance of a history of abortion in aetiology. *Br Med J* 1979; 1: 975-976.
- Evans HJ, O'Riordan ML. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. *Mutat Res* 1975; 31: 135-148.
- Evans J, Townsend L. The induction of ovulation. *Am J Obstet Gynecol* 1976; 125: 321-327.
- Exner T, Rickard KA, Kronenberg H. A sensitive test demonstrating lupus anticoagulant and its behavioural patterns. *Br J Haematol* 1978; 40: 143-151.
- Fantel AG, Shepard TH, Vadheim-Roth C, Stephens TD, Coleman C. Embryonic and fetal phenotypes: Prevalence and other associated factors in a large study of spontaneous abortion. In: Porter IH & Hook EB (eds) *Human Embryonic and Fetal Death*. New York: Academic Press, 1980; 71-87.
- Farquharson RG, Pearson JF, John L. Lupus anticoagulant and pregnancy management. *Lancet* 1984; ii: 228-229.
- Faulk WP, Hsi BL. Immunobiology of human trophoblast membrane antigens. In: Loke YW, Whyte A (eds) *Biology of trophoblast*. Amsterdam, Elsevier 1983, 535-570.
- Faulk WP, Sanderson AR, Temple A. Distribution of MHC antigens in human placental chorionic villi. *Transpl Proc* 1977; 9: 1379-1384.
- Faulk WP, Temple A, Louvins RE, Smith NC. Antigens of human trophoblasts: a working hypothesis for their role in normal and abnormal pregnancies. *Proc Nat Acad Sci USA (B)* 1978; 75: 1947-1951.
- Faulk WP, Temple A. Distribution of beta-2 microglobulin and HLA in chorionic villi of human placentae. *Nature* 1976; 262: 799-802.
- Feldman HA. Congenital toxoplasmosis, at long last *N Eng J Med* 1974; 290: 1138-1140.
- Filicori M, Campaniello E, Michelacci L, Pareschi A, Ferrari P, Bolelli G, Flamigni C. Gonadotropin-releasing hormone (GnRH) analogue

- suppression renders polycystic ovarian disease patients more susceptible to ovulation induction with pulsatile GnRH. *J Clin Endocrinol Metab* 1988; 66: 327-333.
- Firkin BG, Howard MA, Radford SI. Possible relationship between the lupus inhibitor and recurrent abortion in young women. *Lancet* 1980; ii: 366.
- Fishel SB, Cohen J, Fehilly G, Purdy JM, Eurofwalters D, Edwards RG. Factors influencing human embryonic development in vitro. *Ann NY Acad Sci* 1984; 442: 342-356.
- Fishel SB, Edwards RG, Evans CJ. Human chorionic gonadotrophin secreted by preimplantation embryos cultured in vitro. *Science* 1984; 223: 816-818.
- Fitzsimmons J, Wapner RJ, Jackson LG. Repeated pregnancy loss. *Am J Med Genet* 1983; 16: 7-13.
- Fizet D, Bousquet J. Absence of a factor blocking a cellular cytotoxicity reaction in the serum of women with recurrent abortion. *Br J Obstet Gynaecol* 1983; 90: 453-456.
- Fleming AD, Earlich DW, Miller NA, Monif GRG. Successful treatment of maternal septicaemia due to listeria monocytogenes at 28 weeks gestation. *Obstet Gynecol* 1985; 66: 528-530.
- Fleming R, Coutts JRT, Hamilton MP. Evidence against genetic factors causing major loss of embryos. *Br Med J* 1984; 288: 1576.
- Foreman H, Stadel BV, Schlesselman S. Intrauterine device usage and fetal loss. *Obstet Gynecol* 1981; 58: 669-677.
- Forssmann L. Distribution of blood flow in myomatous uteri as measured by locally-injected Zenon. *Acta Obstet Gynecol Scand* 1976; 55: 101-104.
- Foy HM, Kenny GE, Wentworth BB, Johnson WL, Grayston JT. Isolation of mycoplasma hominis, T-strains and cytomegalovirus from the cervix of pregnant women. *Am J Obstet Gynecol* 1970; 106: 635-643.
- Fraga A, Mintz G, Orozco J, Humberto J. Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus. *J Rheumatol* 1974; 1: 293-298.
- Franke H. Zur Aborthäufigkeit bei retroflexio uteri gravidii. *Zeitschrift für Aerztliche Fortbildung* 1973; 67: 1250-1251.
- Freire-Maia N. Abortion, chromosomal aberrations and radiation. *Soc Biol* 1970; 17: 102-106.
- French FE and Bierman JM. Probabilities of fetal mortality. *Public Health Report* 1962; 77:835-847.
- Friberg L, Nordberg GF, Vouk VB (eds). *Handbook on the toxicology of metals*. Amsterdam, Elsevier 1979.
- Fritsch H. Ein fall von völligem schwund der gebärmutterhöhle nach auskratzung. *Zentralblatt für Gynaekologie* 1894; 18: 1337-1342.
- Fritz MA. Inadequate luteal function and recurrent abortion: Diagnosis and treatment of luteal phase deficiency. *Semin Reprod Endocrinol* 1988; 6: 129-143.
- Frydman R, Testart J, Lassalle B, Kerbrat G, Chasseray LE, Papiernik E. Transfert utérin d'un oeuf fécondé in vitro: avortement spontané. *Nouv Presse Med* 1981; 10: 3475-3476.

- Fujikara T, Froehlich LA, Driscoll SG. A simplified anatomic classification of abortions. *Am J Obstet Gynecol* 1966; 95: 902-905.
- Furness ET. Therapeutic abortion, premature labour and cervical incompetence. *Med J Aust* 1983; 24: 604.
- Galbraith GMP, Galbraith RM, Faulk WP. Immunological studies of transferrin and transferrin receptors of human placental trophoblast. *Placenta* 1980; 1: 33-46.
- Galbraith RM, Kantor RRS, Ferrara GB, Ades EW, Galbraith GMP. Differential anatomical expression of transplantation antigens within the normal human placental chorionic villus. *Am J Reprod Immunol* 1981; 1: 331-335.
- Garcia AGP. Intrauterine infection with mumps virus. *Obstet Gynecol* 1980; 56: 756-759.
- Garcia J, Jones GES, Wentz AC. The use of clomiphene citrate. *Fertil Steril* 1977; 28: 707-717.
- Gardiner A, Clarke CA, Cowen J, Finn R, McKendrick OM. Spontaneous abortion and fetal abnormality in subsequent pregnancy. *Br Med J* 1978; 1: 1016-1018.
- Gardner MJ, Altman DG. Confidence intervals rather than P values: estimation rather than hypothesis testing. *Br Med J* 1986; 292: 46-750.
- Garewal G, Sehgal S, Aikat BK, Gupta AN. Cell-mediated immunity in patients with and without a previous history of spontaneous abortions. *Br J Obstet Gynaecol* 1978; 85: 221-224.
- Gatti RA, Yunis EJ, Good RA. Characterisation of a serum inhibitor of MLC reactions. *Clin Exp Immunol* 1973; 13: 427-437.
- Geisler M, Kleinebrecht J. Cytogenetic and histologic analyses of spontaneous abortions. *Hum Genet* 1978; 45: 239-251.
- Gelabert A, Balasch J, Ercilla G, Vanrell JA, Vives J, Gonzales-Merlo J, Castillo R. Abortion may sensitize the mother to HLA antigens. *Tissue Antigens* 1981; 17: 353-356.
- Gerencer M, Drazanic A, Kuvacic I, Tomaskovic Z, Kastelan A. HLA antigen studies in women with recurrent gestational disorders. *Fertil Steril* 1979; 31: 401-404.
- Gilbert GL, Davosen RA, Cole ME, Radord NJ. Midtrimester abortion associated with septicaemia caused by campylobacter jejuni. *Med J Aust* 1981; 1: 585.
- Gill III TJ. Immunological and genetic factors influencing pregnancy and development. *Am J Reprod Immunol Microbiol* 1986; 10:116-120.
- Gill TJ, MacPherson TA, Ho HN, Kunz HW, Hassett AC, Stranick KS, Locker J. Immunological and genetic factors affecting implantation and development in rat and human. In: Gill TJ, Wegmann TG (eds): *Immunoregulation and fetal survival*. Oxford and New York: Oxford University Press, 1986; 137-155.
- Gill TJ, Siew S, Kunz HW. MHC-linked genes affecting development. *J Exp Zool* 1983; 228: 325-345.
- Gilmore DM, McNay MB. Spontaneous fetal loss rate in early pregnancy. *Lancet* 1985, i: 107.

- Glass RH, Golbus MS. Habitual abortion. *Fertil Steril* 1978; 29: 257-265.
- Golbus MS, Loughman WD, Epstein CJ, Halbasch G, Stephens JD, Hall BD. Prenatal genetic diagnosis in 3000 amniocenteses. *N Eng J Med* 1979; 300:157-163.
- Golding J, Butler NR, Newcombe RG. Analysis of completed reproductive histories: a cautionary tale. *J Epidemiol Comm Hlth* 1983; 37: 78-81.
- Goldstein DP. Incompetent cervix in offspring exposed to DES in utero. *Obstet Gynecol* 1978; 52: 733-759.
- Goldzeiher JW. Double blind trial of a progestin in habitual abortion. *JAMA* 1964; 188: 651-654.
- Goldzieher JW, Green JA. The polycystic ovary. 1. Clinical and histological features. *J Clin Endocrinol Metab* 1962; 22: 325-338.
- Goodfellow CF, Taylor PV, Jackson S. Culturing trophoblast from peripheral blood. *Lancet* 1984; ii: 1479.
- Grant A, McBride WG, Moyes JM. Luteal phase defects in abortion. *Int J Fertil* 1959; 4: 323-329.
- Gravett MG, Nelson HP, DeRouen T. Independant associations of bacterial vaginosis and chlamydia trachomatis infection with adverse pregnancy outcome. *JAMA* 1986; 256: 1899-1903.
- Green JE, Dorfmann A, Jones S, Bender SL, Patton L, Schulman JD. Chorionic villus sampling: experience with an initial 940 cases. *Obstet Gynecol* 1988; 71: 208-212.
- Green LK, Harris RE. Uterine anomalies. Frequency of diagnosis and associated obstetric complications. *Obstet Gynecol* 1976; 47: 427-429.
- Greenman GW, Gabrielson MO, Flanders JH, Wessel MA. Thyroid dysfunction in pregnancy. *N Eng J Med* 1962; 267: 426-431
- Gregorini G, Setli G, Remuzzi G. Recurrent abortion with lupus anticoagulant and pre-eclampsia: a common final pathway for two different diseases. *Br J Obstet Gynaecol* 1986; 193: 194-196.
- Greiss JR, Mauzy CH. Genital anomalies in women: An evaluation of diagnosis, incidence and obstetric performance. *Am J Obstet Gynecol* 1961; 82: 330-339.
- Grennan DM, McCormick JN, Wojtacha D. Immunological studies of the placenta in systemic lupus erythematosus. *Ann Rheum Dis* 1978; 37: 129-134.
- Griffith RW, Turkalji I, Braun P. Outcome of pregnancies in mothers given bromocryptine. *Br J Clin Pharmacol* 1978; 5: 227-231.
- Griffiths PD, Campbell-Benzie A, Heath RB. A prospective study of primary cytomegalovirus infection in pregnant women. *Br J Obstet Gynaecol* 1980; 87: 308-314.
- Grönroos M, Honkonen E, Punnönen R. Cervical and serum IgA and serum IgG antibodies to chlamydia trachomatis and herpes simplex virus in threatened abortion: a prospective study. *Br J Obstet Gynecol* 1983; 90: 167-170.
- Guerrero R, Rojas OI. Spontaneous abortion and aging of human ova and spermatozoa. *N Eng J Med* 1975; 293: 573-575.

- Gump DW, Gibson M, Ashikaga T. Lack of association between genital mycoplasmas and infertility. *New Eng J Med* 1984; 310: 937-941.
- Hague WM, Adams J, Reeders S, Jacobs HS. Familial polycystic ovaries. *J Endocrinol* 1986; 108 (suppl) No 108.
- Hall JG, Pauli RM, Wilson KN. Maternal and fetal sequelae of anticoagulation during pregnancy. *Am J Med* 1980; 68: 122-140.
- Hamerton JL, Canning N, Roy M, Smith S. A cytogenetic survey of 14,069 newborn infants. I. Incidence of chromosomal anomalies. *Clin Genet* 1975; 8: 223-243.
- Hansen JW, Streissguth AP, Smith DW. The effects of moderate alcohol consumption during pregnancy on fetal growth and morphogenesis. *J Pediatr* 1978; 92: 457-460.
- Hanshaw JB, Dudgeon JA. Viral diseases of the fetus and newborn. Philadelphia: Saunders, 1978.
- Hanson FW, Zorn EM, Tennant FR, Marianos S, Samuels S. Amniocentesis before 15 weeks gestation: outcome, risks and technical problems. *Am J Obstet Gynecol* 1987; 156: 1524-1531.
- Harger JH, Archer DF, Marchese SG, Muracca-Clemens M, Garver KL. Etiology of recurrent pregnancy losses and outcome of subsequent pregnancies. *Obstet Gynecol* 1983; 62: 574-581.
- Harger JH. Comparison of success and morbidity in cervical cerclage procedures. *Obstet Gynecol* 1980; 56: 543-548.
- Harjulehto T, Aro T, Hovi T, Saxen L. Congenital malformations and oral poliovirus vaccination during pregnancy. *Lancet* 1989; i: 771-772.
- Harlap S, Davies AM. Characteristics of pregnant women reporting previous induced abortion. *Bull WHO* 1975; 52: 149-154.
- Harlap S, Schiono PH, Ramcharan S, Berendes H, Pellegrin F. A prospective study of spontaneous fetal losses after induced abortions. *N Eng J Med* 1979; 301: 677-681.
- Harlap S, Schiono PH, Ramcharan S. Spontaneous fetal losses in women using different contraceptives around the time of conception. *Int J Epidemiol* 1980a; 9: 49-56.
- Harlap S, Schiono PH. Alcohol, smoking and incidence of spontaneous abortions in the first and second trimester. *Lancet* 1980b; ii: 173-176.
- Harlap S, Shiono PH, Ramcharan S. A life table of spontaneous abortions and the effects of age, parity and other variables. In: Porter IH, Hook EB (eds): *Embryonic and fetal death*. New York: Academic Press, 1980; 145-158.
- Harris EN, Boey ML, Mackworth-Young CG, Gharavi AE. Anti-cardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* 1983; ii: 1211-1214.
- Harris EN, Chan JKH, Asherson RA. Thrombosis, recurrent fetal loss and thrombocytopenia: predictive value of the anticardiolipin antibody test. *Arch Intern Med* 1986; 146: 2153-2159.
- Harris EN, Gharavi AE, Loizou S. Cross-reactivity of antiphospholipid antibodies. *J Clin Lab Immunol* 1985a; 16: 1-6.

- Harris EN, Gharavi AE, Patel SP, Hughes GRV. Evaluation of the anticardiolipin antibody test: Report of an international workshop held 4 April 1986; Clin Exp Immunol 1987; 68: 215-222.
- Harris EN, Gharavi AE, Tincani A, Chan JK, Englert H, Mantelli P, Allegro F, Ballestrieri G, Hughes GRV. Affinity purified anticardiolipin and anti-DNA antibodies. J Clin Lab Immunol 1985b; 17: 155-162.
- Harris RE, Lordon RE. The association of maternal lymphocytotoxic antibodies with obstetric complications. Obstet Gynecol 1976; 48: 302-304.
- Harrison HR, Alexander ER, Weinstein L, Lewis M, Nash M, Sim DA. The epidemiology and effects of cervical C. trachomatis and mycoplasmal infections in pregnancy. JAMA 1983; 250: 1721-1727.
- Harrison RF. Treatment of habitual abortion with human chorionic gonadotrophin; results of open and placebo-controlled trials. Eur J Obstet Gynecol Reprod Biol 1985; 20: 159-168.
- Harter CA, Benirschke K. Fetal syphilis in the first trimester. Am J Obstet Gynecol 1976; 124: 705-711.
- Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsunra J, Matsuyama A, Wison C, Yamane JA, Jacobs PA. A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet 1980; 44: 151-178.
- Hassold TJ, Matsuyama IM, Newlands JS, Matsura JD, Jacobs PA. A cytogenetic study of spontaneous abortions in Hawaii. Ann Hum Genet 1978; 41: 443-454.
- Hay D. The diagnosis and significance of minor degrees of uterine abnormality in relation to pregnancy. J Obstet Gynaecol Br Empire 1958; 65: 557-582.
- Hecht F. Unexpected encounters in cytogenetics: repeated abortions and parental chromosome mosaicism may indicate risk of nondisjunction. Am J Genet 1982; 34: 514-515.
- Heinönen PK, Saarikoski S, Pystynen P. Reproductive performance of women with uterine anomalies. Acta Obstet Gynecol Scand 1982; 61: 157-162.
- Hemminki K, Franssila E, Vainio H. Spontaneous abortions among female chemical workers in Finland. Int Arch Occup Env Health 1980a; 45: 123-126.
- Hemminki K, Kyyrönen P, Lindholm ML. Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytotoxic drugs, and other potential hazards in hospital. J Epidemiol Comm Hlth 1985; 39: 141-147.
- Hemminki K, Mutanen P, Saloniemi I. Smoking and the occurrence of congenital malformations and spontaneous abortion: multivariate analysis. Am J Obstet Gynecol 1983; 145: 61-66.
- Hemminki K, Niemi ML, Saloniemi I, Vainio H, Hemminki E. Spontaneous abortions by occupation and social class in Finland. Int J Epidemiol 1980b; 9: 149-153.
- Hensleigh PA, Feinstat T. Corpus luteum dysfunction: serum progesterone levels in diagnosis and assessment of therapy for recurrent and threatened abortion. Fertil Steril 1979; 32: 396-400.

- Herbst AL, Hubby MM, Azizi F, Makii MM. Reproductive and gynecologic surgical experience in DES exposed daughters. *Am J Obstet Gynecol* 1981; 141: 1019-1028.
- Herbst AL, Scully RE. Adenocarcinoma of the vagina in adolescence: A report of seven cases including six clear cell carcinomas (so called mesonephromas). *Cancer* 1970; 25: 745-749.
- Hertig AT, Rock J, Adams EC, Menkin MC. Thirty four fertilised human ova, good, bad and indifferent, recovered from 210 women of known fertility. *Pediatrics* 1959; 23:202-211.
- Hertig AT, Rock J. Searching for early human ova. *Gyn Investig* 1973; 4: 121-138.
- Himmelberger DU, Brown BW, Cohen EN. Cigarette smoking during pregnancy and the occurrence of spontaneous abortion and congenital abnormality. *Am J Epidemiol* 1978; 108: 470-479.
- Hirszfeld L, Zborowski H. Gruppenspezifische beziehungen zwischen mutter und frucht und elektive durchlassigkeit der placenta. *Klin Wochenschr* 1925; 4: 1152.
- Ho C-Y, Aterman K. Infection of the fetus by *Candida* in a spontaneous abortion. *Am J Obstet Gynecol* 1970; 106: 705-710.
- Hofmeyer GJ, Joffe MI, Bezwoda WR, van Iddekinge B. Immunologic investigation of recurrent pregnancy loss and consequences of immunisation with husband's leucocytes. *Fertil Steril* 1987; 48: 681-684.
- Hogue CJR, Cates WJr, Tietze C. The effects of induced abortion on subsequent reproduction. *Epidemiol Rev* 1982; 4: 66-94.
- Holzgreve W, Schonberg SA, Douglas RG. X-chromosome hyperploidy in couples with multiple spontaneous abortions. *Obstet Gynecol* 1984; 63: 237-240.
- Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS. Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. *Br Med J* 1988; 297: 1024-1026.
- Homburg R, Jacobs HS. Etiology of miscarriage in polycystic ovary syndrome. *Fertil Steril* 1989; 51: 196-197.
- Homonai ZI, Paz GF, Weiss JN, David MP. Relation between semen quality and fate of pregnancy: retrospective study on 534 pregnancies. *Int J Androl* 1980; 3: 574-584.
- Honoré LH, Dill FJ, Poland BJ. Placental morphology in spontaneous human abortuses with normal and abnormal karyotypes. *Teratol* 1976; 14: 151-156.
- Honoré LH. The effect of the intrauterine contraceptive device on the prevalence of morphological abnormalities in human spontaneous abortions. *Contraception* 1980; 21: 47-52.
- Hood IC, Desa DJ, Whyte RK. The inflammatory response in candidal chorioamnionitis. *Hum Pathol* 1983; 14: 984-986.
- Horne CHW, Towler CM, Pugh-Humphreys R, Thomson AW, Bohn H. Pregnancy specific β 1-glycoprotein - a production of the syncytiotrophoblast. *Experientia* 1976; 32: 1197-1199.

- Horne HWJr, Kundsins RB, Kosas TS. The role of Mycoplasma infection in human reproductive failure. *Fertil Steril* 1974; 25: 380-389.
- Horstmann DM. Rubella: The challenge of its control. *J Infect Dis* 1971; 123: 640-651.
- Horta JLH, Fernandez JG, Soto de León B, Cortés-Gallegos V. Direct evidence of luteal insufficiency in women with habitual abortion. *Obstet Gynecol* 1977; 49: 705-708.
- Howles CM, Macnamee MC, Edwards RG, Goswamy R, Steptoe PC. Effect of high tonic luteinising hormone on outcome of in-vitro fertilisation. *Lancet* 1986; ii: 521-522.
- Howles CM, Macnamee MC, Edwards RG. Follicular development and early luteal function of conception and non-conception cycles after human in-vitro fertilisation. *Hum Reprod* 1987a; 2: 17-21.
- Hsu LY, Garcia FP, Grossman D. Fetal wastage and maternal mosaicism. *Obstet Gynecol* 1972; 40: 98-103.
- Hsu LY, Perlis TE. United States survey on chromosome mosaicism and pseudomosaicism in prenatal diagnosis. *Prenat Diagn* 1984; 4: 97-130.
- Hubinont CJ, Thomas C, Schwes JF. Luteal function in ectopic pregnancy. *Am J Obstet Gynecol* 1987; 156: 669-674.
- Huisjes HJ. Spontaneous abortion. Edinburgh: Churchill Livingstone, 1984.
- Hull MGR, Savage PE, Bromham DR, Ismail AA, Morris AF. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived from treated and untreated conception cycles. *Fertil Steril* 1982; 37: 355-360.
- Hull MGR. Epidemiology of infertility and polycystic ovarian disease. *Gynecol Endocrinol* 1987; 1: 235-245.
- Hull RG, Harris EN, Morgan SH, Hughes GRV. Anti-Ro antibodies and abortions in women with SLE. *Lancet* 1983; ii: 1138.
- Husslein P, Huber J, Wagen bichler P, Schnedl W. Chromosome abnormalities in 150 couples with multiple spontaneous abortions. *Fertil Steril* 1982; 35: 379-383.
- Infante PF, Wagoner JK, McMichael AJ, Waxweiler RJ, Falk H. Genetic risks of vinyl chloride. *Lancet* 1976; i: 734-735.
- Israel R, March CM. Hysteroscopic incision of the septate uterus. *Am J Obstet Gynecol* 1984; 149: 66-73.
- Israel R, Mishell DR, Stone SC, Thorneycroft IH, Moyer DL. Single luteal phase serum progesterone assay as an indicator of ovulation. *Am J Obstet Gynecol* 1972; 112: 1043-1046.
- Jackson LG. Chorion villus sampling. Newsletter 1988. August - 26. Thomas Jefferson University, Philadelphia.
- Jacobs HS, Porter RN, Eshel A, Craft IL. Profertility uses of LHRH analogues. In : Vickery BH, Nestor JJJr (eds): LHRH and its analogues: contraceptive and therapeutic applications. Part 2. Lancaster, MTP Press 1987, 303-322.
- Jacobs MH, Balasch J, Gonzalez-Merlo JM, Vanrell JA, Wheeler C, Strauss JF, Blasco L, Wheeler JE, Lyttle CR. Endometrial cytosolic and nuclear progesterone receptors in the luteal phase defect. *J Clin Endocrinol Metab* 1987; 64: 472- 475.

- Jacobs PA, Hassold TJ. The origin of chromosome abnormalities in spontaneous abortion. In: Porter IH & Hook EB (eds) Human Embryonic and Fetal Death. Academic Press, New York 1980, 289.
- Jacobs PA, Melville M, Ratcliffe S, Keay AJ, Syme J. A cytogenetic survey of 11,680 newborn infants. *Ann Hum Genet* 1977; 37: 359-376.
- Jacobson CB, Barker RN. Intrauterine diagnosis and management of genetic defects. *Am J Obstet Gynecol* 1967; 99: 795-807.
- Jacoby DR, Olding LB, Oldstone MB. Immunologic regulation of fetal maternal balance. *Adv Immunol* 1984; 35: 157-208.
- Jalali GR, Underwood JL, Mowbray JF. IgG on normal human placenta is bound both to antigen and Fc receptors. *Transpl Proc* 1989; 21: 572-574.
- James WH. Birth ranks of spontaneous abortions in sibships of children affected by anencephaly or spina bifida. *Br Med J* 1978; 1: 72-73.
- James WH. Notes towards an epidemiology of spontaneous abortion. *Am J Hum Genet* 1963; 15: 223-240.
- James WH. On the possibility of segregation in the propensity to spontaneous abortion in the human female. *Ann Hum Genet* 1961; 25: 207-213.
- James WH. Spontaneous abortion and birth order. *J Biosoc Sci* 1974; 6: 23-41.
- Jansen RPS. Spontaneous abortion incidence in the treatment of infertility. *Am J Obstet Gynaecol* 1982; 143: 451-473.
- Jansen RPS. Spontaneous abortion incidence in the treatment of infertility: Addendum on in vitro fertilisation. *Am J Obstet Gynecol* 1982b; 144: 738-739.
- Javert CT. Spontaneous and habitual abortion. New York: McGraw Hill, 1957.
- Jazwinska EC, Kilpatrick DC, Smart GE, Liston WA. Feto-maternal HLA compatibility does not have a major influence on human pregnancy except for lymphocytotoxin production. *Clin Exp Immunol* 1987; 68: 116-122.
- Jeffcoate SL. The endocrine control of ovulation. In: Thompson W, Joyce DN, Newton JR (eds): IVF and Donor insemination. Proceedings of RCOG XIIth Study Group, 1984: 1-9.
- Jenkins DM, Acres MG, Peters J, Riley J. Human chorionic gonadotrophin and the fetal allograft. *Am J Obstet Gynecol* 1972; 114: 13-15.
- Jenkins DM, Hancock KW. Maternal unresponsiveness to paternal histocompatibility antigens in human pregnancy. *Transpl* 1972; 13: 618-619.
- Jennings CL. Temporary submucosal cerclage for cervical incompetence. Report of forty-eight cases. *Am J Obstet Gynecol* 1972; 113: 1097-1102.
- Jensen GK. Leucocyte antibodies in serums of pregnant women. Serology and clinic. *Vox Sang (Basel)* 1962; 7: 454-469.
- Jensen GK. Leucocyte antibodies in serums of pregnant women. *Vox Sang* 1964; 9: 315-322.
- Jensen GK. Transplacental passage of leucocyte agglutinin occurring on account of pregnancy. *Dan Med Bull* 1960; 7: 55-58.
- Jesperperson CS, Littauer J, Sagild U. Measles as a cause of fetal defects. *Acta Paed Scand* 1977; 66: 367-372.

- Jewelewicz R, Khalat S, Neuwirth RS, Vande-Wiele RL. Obstetric complications after treatment of intrauterine synechiae. *Obstet Gynecol* 1976; 47: 701-705.
- Joël CA. New etiologic aspects of habitual abortion and infertility, with special reference to the male factor. *Fertil Steril* 1966; 17: 374-380.
- Johannsen R, Haupt H, Bohn H, Heide K, Seiler FR, Schwick HG. Inhibition of the mixed lymphocyte culture by proteins : mechanism and specificity of the reaction. *Z Immun Forsch Exp Ther* 1976; 152: 280-291.
- Johanssen ED. Progesterone levels in peripheral plasma during the luteal phase of the normal human menstrual cycle measured by a rapid competitive protein binding technique. *Acta Endocrinol (Copenh)* 1969; 61: 592-606.
- Johansson ED. Depression of the progesterone levels in women treated with synthetic gestagens after ovulation. *Acta Endocrinol* 1971; 68: 779-792.
- Johnson A, Godmilow L. Genetic amniocentesis at 14 weeks or less. *Clin Obstet Gynecol* 1988; 31: 345-352.
- Johnson AM, Roberts H, Wetherall B, McDonald PJ, Need JA. Relationship between spontaneous abortion and presence of antibody to toxoplasma gondii. *Med J Aust* 1979; 1: 579-580.
- Johnson P, Brown PJ, Ogbimi AO, Shah LCP. Antigens of the human syncytiotrophoblast microvillous plasma membrane. *Am J Reprod Immunol* 1981a; 1: 83-87.
- Johnson PM, Barnes RMR, Hart CA, Francis WJA. Determinants of immunological responsiveness in recurrent spontaneous abortion. *Transpl* 1984; 38: 280-284.
- Johnson PM, Barnes RMR, Risk JM, Molloy CM, Woodrow JC. Immunogenetic studies of recurrent spontaneous abortions in humans. *Exp Clin Immunogenet* 1985; 2: 77-83.
- Johnson PM, Cheng HM, Molloy CM, Stern CMM, Slade MB. Human trophoblast specific surface antigens identified using monoclonal antibodies. *Am J Reprod Immunol* 1981b; 1: 246-254.
- Johnson PM, Chia KV, Hart CA, Griffith HB, Francis WJA. Trophoblast membrane infusion for unexplained recurrent miscarriage. *Br J Obstet Gynaecol* 1988; 95: 342-347.
- Johnson PM, Molloy CM. Localisation in human term placental bed and amniochorion of cells bearing trophoblast antigens identified by monoclonal antibodies. *Am J Reprod Immunol* 1983; 4: 33-37.
- Jones GES, Delfs E. Endocrine patterns in term pregnancies following abortion. *JAMA* 1951; 146: 1212-1218.
- Jones GES. Luteal phase defects. In: Behrman SJ, Kistner RW (eds). *Progress in infertility*. Second edition. Little, Brown, Boston 1975, 299-324.
- Jones GES. Some newer aspects of the management of infertility. *JAMA* 1949; 141: 1123-1129.
- Jones GES. The luteal phase defect. *Fertil Steril* 1976a; 27: 351-356.
- Jones GS, Aksel S, Wentz AC. Serum progesterone values in luteal phase defects. *Obstet Gynecol* 1974; 44: 26-34.

- Jones GS, Maffezoli RD, Strott CA, Ross GT, Kaplan G. Pathophysiology of reproductive failure after clomiphene-induced ovulation. *Am J Obstet Gynecol* 1970; 108: 847-867.
- Jones HW, Jones GES. Double uterus as an etiological factor of repeated abortion, indication for surgical repair. *Am J Obstet Gynecol* 1953; 65: 323-339.
- Jones HW, Wheelless CR. Salvage of the reproductive potential of women with anomalous development of the Müllerian ducts: 1868-1965-2068. *Am J Obstet Gynecol* 1968; 104: 348-364.
- Jones KL, Smith DW, Streissguth AP, Myrianthopoulos NC. Outcome in offspring of chronic alcoholic women. *Lancet* 1974; i: 1076-1078.
- Jones WR. Immunological aspects of infertility. In: Scott JS, Jones WR (eds): *Immunology of human reproduction*. London, Academic Press 1976: 375-382.
- Jones WS, Mann EB. Thyroid function in human pregnancy. VI. Premature deliveries and reproductive failures of pregnant women with low serum butanol-extractable iodines. Maternal serum TBG and TBPA capacities. *Am J Obstet Gynecol* 1969; 104: 909-914.
- Jones WS. Obstetric significance of female genital anomalies. *Obstet Gynecol* 1957; 10: 113-127.
- Jonker M, van Leeuwen A, van Rood JJ. Inhibition of mixed leucocyte reaction by alloantisera in man. II. Incidence and characterisation of MLC-inhibiting antisera from multiparous women. *Tissue Antigens* 1977; 9: 246-258.
- Joshi SG. Progesterin-regulated proteins of the human endometrium. *Semin Reprod Endocrinol* 1983; 1: 221-236.
- Julkunen M, Apter D, Seppälä M, Stenman UH, Bohn H. Serum levels of placental protein 14 reflect ovulation in nonconception menstrual cycles. *Fertil Steril* 1986a; 45: 47-50.
- Julkunen M, Raikar RS, Joshi SG, Bohn H, Seppälä. Placental protein 14 and progesterin-dependant endometrial protein are immunologically indistinguishable. *Hum Reprod* 1986b; 1: 7-8.
- Kajii T, Ferrier A, Niikawa N, Takahara H, Ohama K, Avirachan S. Anatomic and chromosomal anomalies in 639 spontaneous abortions. *Hum Genet* 1980; 55: 87-98.
- Kajii T, Ferrier A. Cytogenetics of aborters and abortuses. *Am J Obstet Gynecol* 1978; 131: 33-38.
- Kajii T, Niikawa N. Origin of triploidy and tetraploidy in man: 11 cases with chromosome markers. *Cytogenetic Cell Genet* 1977; 18: 109-125.
- Kajii T, Ohama K, Niikawa N, Ferrier A, Avirachan S. Banding analysis of abnormal karyotypes in spontaneous abortion. *Am J Hum Genet* 1973; 25: 539-547.
- Kajii T, Ohama K. Inverse maternal age effect in monosomy X. *Hum Genet* 1979; 51: 147-151.
- Kampelmacher EH, Van Noorle Jansen LM. Listeriose bijmens en dier in Nederland (1958-1977). *Nederlands Tijdschrift voor Geneeskunde* 1979; 123: 557-564.

- Katz AI, Davidson JM, Hayslett JP, Singson E, Lindheimer MD. Pregnancy in women with kidney disease. *Kidney Int* 1980; 18: 192-195.
- Kaufman RH, Adam E, Binder GL, Gerthoffer E. Upper genital tract changes and pregnancy outcome in offspring exposed in utero to diethylstilboestrol. *Am J Obstet Gynecol* 1980; 137: 299-308.
- Kaufman RH, Binder GL, Gray PM, Adam E. Upper genital tract changes associated with exposure in utero to diethylstilboestrol. *Am J Obstet Gynecol* 1977; 128: 51-59.
- Kaufman RH, Noller K, Adam E, Irwin J, Gray J, Jeffries JA, Hilton J. Upper genital tract abnormalities of pregnancy outcome in DES exposed progeny. *Am J Obstet Gynecol* 1984; 148: 973-984.
- Kaufman RH. DES exposure and reproductive performance. In: Bennett MJ, Edmonds DK (eds). *Spontaneous and recurrent abortion*. Oxford: Blackwell Scientific Publications, 1987; 130-148.
- Keller DW, Wiest WG, Askin FB, Johnson LW, Strickler RC. Pseudocorpus luteum deficiency: A local defect of progesterone action on endometrial stroma. *J Clin Endocrinol Metab* 1979; 48: 127-132.
- Keller PJ, Soyka E. Abortifacient effect of intrauterine contraceptive device? *Gynecol Obstet Invest* 1978; 9: 219-221.
- Kennedy TG. Prostaglandins and the endometrial vascular permeability changes preceeding blastocyst implantation and decidualisation. *Prog Reprod Biol* 1980; 7: 234-243.
- Kerin JF, Warnes GM, Quinn P, Kirby C, Jeffrey R, Mathews CD, Seamark RF, Texler K, Antonas B, Cox LW. In vitro fertilisation and embryo transfer program. *J In Vitro Fertil Embryo Transfer* 1984; 1: 63-71.
- Kharazzi M, Potashnik G, Goldsmith JR. Reproductive effects of dibromochloropropane. *Isr J Med Sci* 1980; 16: 403-406.
- Khong T Y, De Wolf F, Robertson W B, Brosens I. Inadequate maternal vascular response in pregnancies complicated by preeclampsia and by small for gestational age infants. *Br J Obstet Gynaecol* 1986; 93: 1049-1059.
- Khong T Y, Liddell H S, Robertson W B. Defective haemochorial placentation as a cause of miscarriage: a preliminary study. *Br J Obstet Gynaecol* 1987; 94: 649-655.
- Kilpatrick DC. A case of materno-fetal histocompatibility- implications for leucocyte transfusion treatment for recurrent abortions. *Scott Med J* 1984; 29: 110-112.
- Kim HJ, Hsu LY, Paciuc S, Cristian S, Quintana A, Hirschorn K. Cytogenetics of fetal wastage. *N Eng J Med* 1975; 293: 844-847.
- Kimball AC, Kean BH, Fuchs F. The role of toxoplasmosis in abortion. *Am J Obstet Gynecol* 1971; 111: 219-226.
- Kinney JS, Anderson LJ, Farrar J, Strikas RA, Kumar ML, Kliegman RM, Sever JL, Hurwitz ES, Sikes RK. Risk of adverse outcomes of pregnancy after human parvovirus B19 infection. *J Infect Dis* 1988; 157: 663-667.
- Kirby DRS, Billington WD, Bradbury S, Goldstein DJ. Antigen barrier of the mouse placenta. *Nature* 1964; 204: 549-549.
- Kirby DRS, Billington WD, James DA. Transplantation of eggs to the kidney and uterus of immunised mice. *Transpl* 1966; 4: 713-718.

- Klee JG. Undiagnosed Wilson's disease as a cause of unexplained miscarriage. *Lancet* 1979; ii: 423.
- Kline J, Levin B, Shrout P, Stein Z, Susser M, Warburton D. Maternal smoking and trisomy among spontaneously aborted conceptions. *Am J Hum Genet* 1983; 35: 421-431.
- Kline J, Shrout P, Stein Z, Susser M, Warburton D. Drinking during pregnancy and spontaneous abortion. *Lancet* 1980; ii: 176-180.
- Kline J, Stein Z, Susser M, Warburton D. Fever during pregnancy and spontaneous abortion. *Am J Epidemiol* 1985; 121: 832-842.
- Kline J, Stein Z, Susser M, Warburton D. Induced abortion and spontaneous abortion: no connection? *Am J Epidemiol* 1978; 107: 290-298.
- Kline J, Stein Z, Susser M, Warburton D. Induced abortion and the chromosomal characteristics of subsequent miscarriages (spontaneous abortions). *Am J Epidemiol* 1986; 123:1066-1079.
- Kline J, Stein Z, Susser M, Warburton D. Smoking: a risk factor for spontaneous abortion. *N Eng J Med* 1977; 297: 793-796.
- Kline J, Stein Z. Epidemiology of chromosomal anomalies in spontaneous abortion: prevalence, manifestation and determinants. In: Bennett MJ, Edmonds DK (eds). *Spontaneous and recurrent abortion*. Oxford: Blackwell Scientific Publications, 1987; 29-50.
- Klinger A. Demographic consequences of the legalisation of induced abortion in Eastern Europe. *Int J Obstet Gynecol* 1970; 8: 680-691.
- Klinger HP, Glasser M, Kava HW. Contraceptives and the conceptus. 1. Chromosome abnormalities of the fetus and neonate related to maternal contraceptive history. *Obstet Gynecol* 1976; 48: 40-48.
- Klopper AI, MacNaughton MC. Hormones in recurrent abortion. *J Obstet Gynaecol Br Commonw* 1965; 72: 1022-1028.
- Knight AH, Rhind EG. Epilepsy and pregnancy: A study of 153 pregnancies in 59 patients. *Epilepsia* 1975; 16: 99-104.
- Knill-Jones RP, Newman BJ, Spence AA. Anaesthetic practice and pregnancy: Controlled survey of women anaesthetists in the United Kingdom. *Lancet* 1972; i: 1326-1328.
- Kohn G, Ornoy A, Ben-Tsur Z, Sadovsky E, Cohen MM. Successive spontaneous abortions with diverse chromosomal aberrations in human translocation heterozygotes. *Teratol* 1976; 12: 283-290.
- Köller O, Eikhorn SN. Late sequelae of induced abortion in primigravidae. *Acta Obstet Gynecol Scand* 1977; 56: 311-317.
- Kölmodin-Hedman B, Hedström L, Gröqvist B. Menopausal age and spontaneous abortion in a group of women working in a Swedish steel works. *Scand J Soc Med* 1982; 10: 17-22.
- Komlos L, Halbrecht I. Repeated abortions and histocompatibility antigens: can HLA antigen restricted gene dose effects influence the fetomaternal relationship? *Med Hypotheses* 1979; 5: 901-908.
- Komlos L, Zamir R, Joshua H, Halbrecht I. Common HLA antigens in couples with repeated abortions. *Clin Immunol Immunopathol* 1977; 7:330-335.

- Kowen DE, Hanslo DH, Botha PL, Davet DA. Incidence of aerobic and anaerobic infection in women with incomplete abortion. *S Afr Med J* 1979; 55: 129-132.
- Krieg H, Kasper K. Zur frage der ABO-inkompatibilitat als Abortursache. *Geburtschilfe und Frauenheilkunde* 1967; 27: 679-689.
- Kriel RL, Gates GA, Wulff H, Powell N, Poland JD, Chin TDY. Cytomegalovirus isolations associated with pregnancy wastage. *Am J Obstet Gynecol* 1970; 106: 885-892.
- Kullander S, Källén B. A prospective study of smoking and pregnancy. *Acta Obstet Gynecol Scand* 1971; 50: 83-94.
- Kundsin RB, Ampola M, Streeter S, Neurath P. Chromosomal aberrations induced by T-strain mycoplasmas. *J Med Genet* 1971; 8: 181-187.
- Kundsin RB, Driscoll SG. Mycoplasmas and human reproductive failure. *Surg Gynecol Obstet* 1970; 131: 89-92.
- Kurppa K, Holmberg P, Nurminen T, Saxen L. Birth defects and video display terminal exposure during pregnancy. *Scand J Work Environ Health* 1985; 11: 353-356.
- Lancet editorial. Perinatal listeriosis. *Lancet* 1980; i: 911.
- Lancet editorial. The Shirodkar stitch. *Lancet* 1977; ii: 552-553.
- Landgren B, Uden A, Diczfalusy E. Hormonal profile of the cycle in 68 normally menstruating women. *Acta Endocrinol (Copenh)* 1980; 94: 89-98.
- Laurence KM, Roberts CJ. Spina bifida and anencephaly: are miscarriages a possible cause? *Br Med J* 1977; 3: 361-362.
- Lauritsen JG, Grunnet N, Jensen OM. Materno-fetal ABO incompatibility as a cause of spontaneous abortion. *Clin Genet* 1975; 7: 308-316.
- Lauritsen JG, Jørgensen J, Kissmeyer-Nielsen F. Significance of HLA and blood group incompatibility in spontaneous abortion. *Clin Genet* 1976a; 575-582.
- Lauritsen JG. Aetiology of spontaneous abortion. A cytogenetic and epidemiological study of 288 abortuses and their parents. *Acta Obstet Gynaecol Scand* 1976; 53: 1-29.
- Lauritsen JG. Genetic aspects of spontaneous abortion. *Dan Med Bull* 1977; 24: 169-189.
- Lauritsen JG. The significance of oral contraceptives in causing chromosome anomalies in spontaneous abortions. *Acta Obstet Gynecol Scand* 1975; 54: 261-264.
- Lee CS. Luteal phase defects. *Obstet Gynecol Surv* 1987; 42: 267-274.
- Lee RV. The problems of malaria and toxoplasmosis. *Clin Perinatol* 1988; 15: 351-363.
- Lenton EA, Landgren B, Sexton L. Normal variation in the length of the luteal phase of the menstrual cycle: Identification of the short luteal phase. *Br J Obstet Gynaecol* 1984; 91: 685-689.
- Lenton EA, Neal LM, Sulaiman R. Plasma concentrations of human chorionic gonadotrophin from the time of implantation until the second week of pregnancy. *Fertil Steril* 1982; 37: 773-778.

- Leridon H. Facts and artefacts in the study of intrauterine mortality: a reconsideration from pregnancy histories. *Population studies* 1976; 30: 319-335.
- Leridon H. Human fertility: the basic components. Chicago: University of Chicago Press, 1977.
- Levine MM, Edsall G, Bruce-Chwatt LJ. Live virus vaccines in pregnancy, risks and recommendations. *Lancet* 1974; 2: 34-36.
- Levine P, Koch EA. The rare human isoagglutinin anti-Tj^a and habitual abortion. *Sci* 1954; 120: 239-241.
- Levy C, Robel P, Gautray JP, Brux J de, Verma U, Descomps B, Baulieu EE. Estradiol and progesterone receptors in human endometrium: normal an abnormal menstrual cycles and early pregnancy. *Am J Obstet Gynecol* 1980; 136: 646-651.
- Leyendecker G. The pathophysiology of hypothalamic ovarian failure. *Eur J Obstet Gynecol Reprod Biol* 1979; 9: 175-186.
- Liestöl K. Menarcheal age an spontaneous abortion: a causal connection? *Am J Epidemiol* 1980; 111: 753-758.
- Lilford RJ. Chorion villus biopsy. *Arch Dis Child* 1985; 60: 897-899.
- Lindenbaum RH, Bobrow M. Reciprocal translocations in man. *J Med Genet* 1975; 12: 29-43.
- Lippman-Hand A, Vekemans M. Balanced translocations among couples with two or more spontaneous abortions. Are males and females equally likely to be carriers? *Hum Genet* 1983; 63: 252-257.
- Lipshitz J. Cerclage in the treatment of incompetent cervix. *S Afr Med J* 1975; 49: 2013-2018.
- Little RE. Moderate alcohol use during pregnancy and decreased infant birthweight. *Am J Publ Hlth* 1977; 67: 1154-1156.
- Livingstone JE, Poland BJ. A study of spontaneously aborted twins. *Teratol* 1980; 21: 139-148.
- Lockshin MD, Druzin ML, Goei S, Quamar T, Magid MS, Jovanovic L, Ferenc M. Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. *N Eng J Med* 1985; 313: 152-156.
- Lockshin MD, Quamar T, Druzin ML, Goei S. Antibody to cardiolipin, lupus anticoagulant and fetal death. *J Rheumatol* 1987; 14: 259-262.
- Lockwood CJ, Reece EA, Romero R, Hobbins JC. Anti-phospholipid antibody and pregnancy wastage. *Lancet* 1986; ii: 742-743.
- Loke YW, Carter DB, Whyte A, Brook SS, Day S. Electrophoretic mobilities of human trophoblast cells and lymphocytes from pregnant women. *J Reprod Immunol* 1980a; 2: 45-52.
- Loke YW, Wilson DV, Borland R. Localisation of human chorionic gonadotrophin in monolayer cultures of trophoblast cells by mixed agglutination. *Am J Obstet Gynecol* 1972; 113: 875-879.
- Loke YW. Human trophoblast antigens. In: Stern C (ed): *Immunology of pregnancy and its disorders*. Lancaster, Kluwer Academic Publishers 1989, 61-89.

- Lolis D, Tzigounis V, Michalas S, Koumentakou E, Kaskerelis D. Toxoplasma antibodies and spontaneous abortion. *Int J Gynecol Obstet* 1978; 15: 299-301.
- Longo LD. Carbon monoxide effects on oxygenation of the fetus in utero. *Science* 1976; 194: 523-525.
- Lopata A. Successes and failures in human in vitro fertilisation. *Nature* 1980; 288: 642-643.
- Lubbe WF, Butler WS, Palmer SJ, Liggins GC. Fetal survival after prednisolone suppression of maternal lupus anticoagulant. *Lancet* 1983; i: 1361-1363.
- Lubbe WF, Butler WS, Palmer SJ, Liggins GC. Lupus anticoagulant in pregnancy. *Br J Obstet Gynaecol* 1984; 91: 357-363.
- Lubbe WF, Liggins GC. Lupus anticoagulant and pregnancy. *Am J Obstet Gynecol* 1985; 135: 322-327.
- Lubbe WF, Liggins GC. Role of lupus anticoagulant and autoimmunity in recurrent pregnancy loss. *Sem Reprod Endocrinol* 1988; 6: 181-190.
- Lunenfeld B. In: Greenblatt RB (ed): *Induction of ovulation*. Philadelphia, Lea & Febiger 1979, 9-34.
- MacDonald RR, Goulden R, Oakey RE. Cervical mucus, vaginal cytology and steroid secretion in recurrent abortion. *Obstet Gynecol* 1972; 40: 394-402.
- MacGregor JD, Avery JG. Malaria transmission and fetal growth. *Br Med J* 1974; 3: 433-436.
- Machin GA, Crolla JA. Chromosome constitution of 500 infants dying during the perinatal period. *Humangenetik* 1974; 23: 183-198.
- Mackay C. The alleged reproductive hazards of VDUs. *Work and Stress* 1987; 1: 49-57.
- MacKenzie WE, Holmes DS, Newton JR. Spontaneous abortion rate in ultrasonographically viable pregnancies. *Obstet Gynecol* 1988; 71: 81-83.
- MacLachlan NA, Rooney DE, Coleman D, Rodeck CH. Prenatal diagnosis: early amniocentesis or chorionic villus sampling. *Contemp Rev Obstet Gynaecol* 1989; i: 173-180.
- MacLeod AM, Mason RJ, Stewart KN, Power DA, Shewan WG, Edward N, Catto GRD. Fc-receptor blocking antibodies develop after blood transfusions and correlate with good graft outcome. *Transpl Proc* 1983; 15: 1019-1021.
- MacLeod AM, Mason RJ, Stewart KN, Power DA, Shewan WG, Edward N, Catto GRD. Possible mechanisms of action of transfusion effect in renal transplantation. *Lancet* 1982; ii: 468-470.
- Macnamee MC, Howles CM, Edwards RG. Pregnancies after high tonic LH is reduced by long-term treatment with GnRH agonists. *Hum Reprod* 1987; 2: 569-571.
- MacNaughton MC. Hormone assays in early pregnancy. In: Klopper A (ed) *Plasma hormone assays in evaluation of fetal wellbeing*. Edinburgh: Churchill Livingstone, 1976; 36-47.
- MacNaughton MC. *Listeria monocytogenes* in abortion. *Lancet* 1962; ii: 484
- MacNaughton MC. The probability of recurrent abortion. *J Obstet Gynaecol Br Commonw* 1964; 71:784.

- Mall EP, Meyer AW. Studies on abortuses: a survey of pathologic ova in the Carnegie embryological collection. In: Contributions to embryology Vol 12. Publications of the Carnegie Institution 1921; 1-364.
- Malone LJ, Ingersoll FM. Myomectomy in infertility. In: Behrman SJ, Kistner RW (eds) Progress in infertility. Boston, Little Brown & Co., 1975, 80-89..
- Malpas P. A study of abortion sequences. J Obstet Gynaecol Br Empire 1938; 45: 932-949.
- Mangan CE, Borow L, Burtneitt-Rubin MM, Egan V, Ginntoli RL, Mikuta JJ. Pregnancy outcome in 98 women exposed to DES in utero, their mothers, and unexposed siblings. Obstet Gynecol 1982; 59: 315-319.
- Mann EC, McLaren WD, Hayt DB. The physiology and significance of the uterine isthmus. Am J Obstet Gynecol 1961; 81: 209-222.
- Manson MM, Logan WPD, Loy RM. Rubella and other virus infections during pregnancy. Reports on public health and medical subjects, No.101. HMSO, London 1960.
- March CM, Israel R. Gestational outcome following hysteroscopic lysis of adhesions. Fertil Steril 1981; 36: 455-459.
- Marelli G, Vignali M, Cenzuales S, Busacca M. Blood transfusions in habitual abortion. Fertil Steril 1986; 46: 1167.
- Marshall J. Thermal changes in the normal menstrual cycle. Br Med J 1963; 1: 102-104.
- Martin DH, Koutsky L, Eschenbach DA. Prematurity and perinatal mortality in pregnancies complicated by chlamydia trachomatis infection. JAMA 1982; 247: 1585-1588.
- Martin L. What roles are fulfilled by uterine epithelial components in implantation. Prog Reprod Biol 1980; 7: 54-59.
- Mason RJ, Power DA, McIntyre JA. Differential expression of trophoblast lymphocyte cross-reactive (TLX) antigens on T and B lymphocytes. Am J Reprod Immunol Microbiol 1986; 10: 151-155.
- Mathur S, Gust JM, Williamson HO, Fudenberg HH. Antigenic cross-reactivity of sperm and T-lymphocytes. Fertil Steril 1980; 34: 469-476.
- Matsunago E, Ito S. Blood groups and fertility in a Japanese population, with special reference to intrauterine selection due to maternal-fetal incompatibility. Ann Hum Genet 1958; 22: 111-131.
- Mausher SJ, Garcia JE. In: Jones HW, Jones GES, Hodgen GD (eds). Pregnancy and its outcome in in vitro fertilisation-Norfolk. Baltimore, Williams & Wilkins 1986, 238-259.
- Maxwell D, Lilford RJ, Czepulkowski B, Heaton D, Coleman D. Transabdominal chorionic villus sampling. Lancet 1986; i: 123-126.
- Maynard PV, Symonds EM, Johnson J, Baker PN, Sant-Cassia LJ, Selby C. Nuclear progesterone uptake by endometrial tissue in cases of subfertility. Lancet 1983; ii: 310-312.
- McAnulty JH, Metcalfe T, Ueland K. Cardiovascular disease. In: Burrow GN, Ferris TF (eds): Medical complications during pregnancy. Philadelphia, WB Saunders 1982, 180-203.

- McArthur JW, Ingersoll FM, Worcester J. The urinary excretion of interstitial-cell and follicle stimulating hormone activity by women with diseases of the reproductive tract. *J Clin Endocrinol Metab* 1958; 18: 1202-1215.
- McDonald AD. Seasonal distribution of abortions. *Br J Prev Soc Med* 1971; 25: 222-224.
- McDonald IA. Cervical cerclage. *Clin Obstet Gynecol* 1980; 7: 461-479.
- McDonald IA. Cervical incompetence as a cause of spontaneous abortion. In: Bennett MJ, Edmonds DK (eds). *Spontaneous and recurrent abortion*. Oxford: Blackwell Scientific Publications, 1987; 168-192.
- McDonald IA. Suture of the cervix for inevitable miscarriage. *J Obstet Gynaecol Br Empire* 1957; 63: 346-352.
- McDonough PG. Progesterone therapy: Benefit versus risk. *Fertil Steril* 1985; 44: 13-16.
- McFadyen IR. Missed abortion, and later spontaneous abortion in pregnancies clinically normal at 7-12 weeks. *Eur J Obstet Gynecol Reprod Biol* 1985; 20: 381-384.
- McIntyre JA, Faulk WP, Nichols-Johnson VR, Taylor CG. Immunologic testing and immunotherapy in recurrent spontaneous abortion. *Obstet Gynecol* 1986; 67: 169-173.
- McIntyre JA, Faulk WP. Allotypic trophoblast lymphocyte cross-reactive (TLX) surface antigens. *Hum Immunol* 1982a; 4: 27-35.
- McIntyre JA, Faulk WP. HLA and the generation of diversity in human pregnancy. *Placenta* 1982b; 1(suppl 4): 11-16.
- McIntyre JA, Faulk WP. Recurrent spontaneous abortion in human pregnancy: results of immunogenetical, cellular and humoral studies. *Am J Reprod Immunol* 1983; 4: 165-170.
- McIntyre JA, Faulk WP. Trophoblast modulation of maternal allogeneic recognition. *Proc Nat Acad Sci USA* 1979a; 76: 4029-4032.
- McIntyre JA, Hsi B, Faulk WP, Klopfer A, Thomson R. Immunological studies of the human placenta: functional and morphological analysis of pregnancy associated plasma protein A (PAPP-A). *Immunol* 1981; 44: 577-583.
- McIntyre JA, McConnachie PR, Taylor CG, Faulk WP. Clinical, immunologic and genetic definitions of primary and secondary recurrent spontaneous abortions. *Fertil Steril* 1984; 42: 849-855.
- McLaren A. Antigenic disparity: does it affect placental size, implantation or population genetics? In: Edwards RG, Howe CWS, Johnson MH (eds): *Immunobiology of trophoblast*. Cambridge University Press, 1975; 225-273.
- McLaughlin PJ, Cheng HM, Slade MB, Johnson PM. Expression on cultured human tumour cells of placental trophoblast membrane antigens and placental alkaline phosphatase by monoclonal antibodies. *Int J Cancer* 1982; 30: 21-26.
- McNatty KP, Sawers RS. Relationship between the endocrine environment within the Graafian follicle and the subsequent rate of progesterone secretion by human granulosa cells in vitro. *J Endocrinol* 1975; 66: 391-400.

- McNay MB, Whitfield CR. Amniocentesis. *Br J Hosp Med* 1984; 31: 406-417.
- McRae MA, Blasco L, Lyttle CR. Serum hormones and their receptors in women with normal and inadequate corpus luteum function. *Fertil Steril* 1984; 42: 58-63.
- McVerry BA, Spearing R, Smith A. SLE anticoagulant: Transient inhibition by high dose immunoglobulin infusion. *Br J Haematol* 1985; 61: 579-580.
- Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol* 1954; 7: 320-338.
- Menge AC, Beer AE. The significance of human leukocyte antigen profiles in human infertility, recurrent abortion and pregnancy disorders. *Fertil Steril* 1985; 43: 693-695.
- Menge AC, Fleming CH. Detection of sperm antigens on mouse ova and early embryos. *Dev Biol* 1978; 63: 111-117.
- Menge AC, Medley NE, Mangione CM, Dietrich JW. The incidence and influence of antisperm antibodies in infertile human couples on sperm-cervical mucus interactions and subsequent fertility. *Fertil Steril* 1982; 38: 439-446.
- Mennutti M. Prenatal Diagnosis - Advances bring new challenges. *N Eng J Med* 1989; 320: 661-663.
- Mennutti MT, Jingeleski S, Schwartz RH, Mellman WJ. An evaluation of cytogenetic analysis as a primary tool in the assessment of recurrent pregnancy wastage. *Obstet Gynecol* 1978; 52: 308-313.
- Meyer JG. The teratological effects of anticonvulsants and the effects on pregnancy and birth. *Eur Neurol* 1973; 10: 179-181.
- Michelmann HW, Mettler L. Cytogenetic investigations on human oocytes and early human embryonic stages. *Fertil Steril* 1985; 43: 320-322.
- Michels VV, Medrano C, Nenne VL, Riccardi VM. Chromosome translocations in couples with multiple spontaneous abortions. *Am J Hum Genet* 1982; 34: 507-513.
- Mikkelsen M. Chromosome analysis on chorionic villi. *Clin Obstet Gynaecol* 1987; 1: 533-546.
- Mikkelsen M. Cytogenetic findings in first trimester chorionic villi biopsies: A collaborative study. In: Fraccaro G, Simoni G, Brambati B (eds). *First trimester Fetal Diagnosis*. Berlin: Springer-Verlag, 1985, 109-120.
- Mikkelsen M. Downs syndrome. Current state of epidemiology. In: Bonne-Tamir B (ed) *Human Genetics*; New York, Alan R Liss 1982, 297-307.
- Miller JF, Williamson E, Glue J, Gordon YG, Grudzinskas JG, Sykes A. Fetal loss after implantation. *Lancet* 1980; ii: 554-556.
- Miller JR, Poland BJ. Some epidemiological data on 902 spontaneous abortions. In: Boué A, Thibault C. *Chromosomal errors in relation to reproductive failure*. INSERM, Paris 1973; 289-295..
- Miodovnik M, Lavin JP, Knowles HC, Holroyde J, Stys SJ. Spontaneous abortion among insulin-dependant diabetic women. *Am J Obstet Gynecol* 1984; 150: 372-376.

- Miodovnik M, Mimoumi F, Siddiqi TA, Tsang RC. Periconceptual metabolic status and risk for spontaneous abortion in insulin-dependant diabetic pregnancies. *Am J Perinatol* 1988; 4: 368-373.
- Mittal KM, Mickey MR, Singal PP, Terasaki PI. Serotyping for homotransplantation XVIII: Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* 1969; 6: 904-912.
- Moloney MD, Bulmer JN, Scott JS, Need JA, Pattison NS. Maternal immune responses and recurrent miscarriage. *Lancet* 1989; i: 45-46.
- Moor RM, Trounson AO. Hormonal and follicular factors affecting maturation of sheep oocytes in vitro and their subsequent developmental capacity. *J Reprod Fertil* 1977; 49: 101-109.
- Mortimer PP, Cohen BJ, Buckley MM, Craddock-Watson JE, Ridelagh MKS, Burkhardt F, Schilt U. Human parvovirus and the fetus. *Lancet* 1985; ii: 1012.
- Morton H, Rolfe B, Clunie GJA, Anderson MJ, Morrison J. An early pregnancy factor detected in human serum by the rosette inhibition test. *Lancet* 1977; i: 394-397.
- Morton H. Early pregnancy factor; a link between fertilisation and immuno - modulation. *Aust J Biol Sci* 1984; 37: 393-407.
- Mottironi VD, Hook EB, Willey AM, Porter IH, Swift RV, Hatcher NH. Decreased HLA heterogeneity in parent of children with Down syndrome. *Am J Hum Genet* 1983; 35: 1289-1296.
- Moulinier J, Merle MC, Mesnier F, Servantier X. Les anticorps anti-tissulaires de la femme immunisee anti-Rh. In: *Proceedings of XII International Congress on Blood Transfusion, Moscow 1970*; 214-215.
- Mourant AE, Kopeć AC, Domaniewska-Sobczak K. *Blood groups and diseases*. Oxford University Press 1978.
- Mouzon J, Belaisch-Allart J, Cohen J, Dubuisson JB, Guichard A, Parinaud J, Bachelot A, Chalais JJ. Dossier FIVNAT: analyse des resultats 1987. *Contr Fertil Sex* 1988; 16: 599-615.
- Mowbray JF, Gibbings C, Liddell H, Reginald PW, Underwood JL, Beard RW. Controlled trial of treatment of recurrent spontaneous abortion with paternal cells. *Lancet* 1985; i: 941-943.
- Mowbray JF, Gibbings CR, Sidgwick AS, Ruszkiewicz M, Beard RW. Effects of transfusion in women with recurrent spontaneous abortion. *Transplant Proc* 1983; 15: 896-899.
- Mowbray JF, Underwood JL, Michel M, Forbes PB, Beard RW. Immunisation with paternal lymphocytes in women with recurrent miscarriage. *Lancet* 1987; 2: 679-680.
- Mowbray JF. A controlled trial of immunotherapy with paternal cells in recurrent spontaneous abortion. In: Gill TJ, Wegmann TG (eds): *Immunoregulation and fetal survival*. Oxford University Press, 1987: 300-308.
- Mowbray JF. In: Beard RW, Sharp F (eds): *Early pregnancy loss: Mechanisms and treatment*. Springer-Verlag, 1988; 330.
- MRC Working Party. An assessment of the hazards of amniocentesis. *Br J Obstet Gynaecol* 1978; 85: 1-41.

- MRC/RCOG Working Party on cervical cerclage. Interim Report. *Br J Obstet Gynaecol* 1988; 95: 437-445.
- Murphy J, Mulcahy R. Cigarette smoking and spontaneous abortion. *Lancet* 1978; i: 917-920.
- Musich JR, Behrman SJ. Obstetric outcome before and after metroplasty in women with uterine anomalies. *Obstet Gynecol* 1978; 52: 63-66.
- Nahmias AJ, Josey WE, Naib ZM, Freeman MG, Fernandez RJ, Wheeler JH. Perinatal risk associated with maternal herpes simplex virus infections. *Am J Obstet Gynecol* 1971; 110: 825-837.
- Naib ZM, Nahmias AJ, Josey WE, Wheeler JH. Association of maternal genital herpetic infection with spontaneous abortion. *Obstet Gynecol* 1970; 35: 260-263.
- Naito S, Mickey MR, Ebbin AJ, Strauss J, Terasaki PI. Maternal-fetal incompatibility. II. Direct cross-match studies on 38 children with birth defects. In: WF Bodmer, JR Batchelor, JG Bodmer (eds): *Histocompatibility testing*: Munksgaard Copenhagen, 1970; 489-494.
- Naples JD, Batt RE, Sadigh H. Spontaneous abortion rate in patients with endometriosis. *Obstet Gynecol* 1981; 57: 509-512.
- Naumoff N, Shook DM. Abortion and low thyroid reserve. *Int J Fertil* 1963; 8: 811-813.
- Naylor AF, Warburton D. Sequential analysis of spontaneous abortion. II. Collaborative study data show that gravidity determines a very substantial rise in risk. *Fertil Steril* 1979; 31: 282-286.
- Naylor AF. Sequential aspects of spontaneous abortion: maternal age, parity and pregnancy compensation artefact. *Soc Biol* 1974; 21: 195-204.
- NICHD National Registry for Amniocentesis Study Group. Midtrimester amniocentesis for prenatal diagnosis: safety and accuracy. *JAMA* 1976; 236: 1471-1476.
- Nicholas NS, Panayi GS, Murphy J, Pitzalis C. Human retroplacental sera inhibit the expression of Class II major histocompatibility antigens. *J Reprod Immunol* 1986; 9: 95-102.
- Nicholas NS, Panayi GS. Inhibition of interleukin 2 production by retroplacental sera: a possible mechanism for human allograft survival. *Am J Reprod Immunol Microbiol* 1985; 9: 6-11.
- Nickson DA, Sutcliffe RG. A search for antibodies in term maternal sera to solubilised syncytiotrophoblast surface components. *J Reprod Immunol* 1986; 9: 303-312.
- Nicolaides KH, Soothill PW, Rosevear SK. Transabdominal placental biopsy. *Lancet* 1987; ii: 855-856.
- Nielsen J, Sillesen J. Incidence of chromosome aberrations among 11,148 newborn children. *Humangenetik* 1975; 30: 1-12.
- Nielsen J. Large Y chromosome (Yq+) and increased risk of abortion. *Clin Genet* 1978; 13: 415-416.
- Nishimura H, Takano K, Tanimura T, Yasuda M. Normal and abnormal development of human embryos: first report on the analysis of 1213 embryos. *Teratol* 1968; 1: 281-290.

- Niswander KR, Gordon M (eds). The women and their pregnancies. Philadelphia: WB Saunders Co, 1972; 389.
- Nixon GS, Helsby CA, Gordon H, Hytten FE, Renson CE. Pregnancy outcome in female dentists. *Br Dental J* 1979; 146: 39-42.
- Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Fertil Steril* 1950; 1: 3-25.
- Nymand G, Heron I, Jensen GK, Lundsgaard A. Cytotoxic antibodies in serum of pregnant women at delivery. *Acta Path Microbiol Scand* 1971; 79: 595-598.
- Nymand G. Complement-fixing and lymphocytotoxic antibodies in serum of pregnant women at delivery. *Vox Sang* 1974; 27: 322-337.
- O'Neill C, Gidley-Baird AA, Pike IL, Porter RN, Sinosich MJ, Saunders DM. Maternal blood platelet physiology and luteal phase endocrinology as a means of monitoring pre and post implantation embryo viability following IVF. *J In Vitro Fertil Embryo Trans* 1985; 2: 87-93.
- O'Neill C. Examination of the causes of early pregnancy-associated thrombocytopenia in mice. *J Reprod Fertil* 1985b; 75: 567-577.
- O'Neill C. Partial characterisation of the embryo-derived platelet-activating factor in mice. *J Reprod Fertil* 1985c; 75: 375-380.
- Obel EB. Risk of spontaneous abortion following legally induced abortion. *Acta Obstet Gynecol Scand* 1980; 59: 131-135.
- Ober CL, Martin AO, Simpson JL, Hanck WW, Amos DB, Kostyn DD, Fotino M, Allen FHJ. Shared HLA antigens and reproductive performance among Hutterites. *Am J Hum Genet* 1983; 35: 994-1004.
- Ochslager FK. Listeriosis as a possible cause of abortion. Report of a case. *Obstet Gynecol* 1960; 16: 595-596.
- Oechsli FW. Studies of the consequences of contraceptive failure. Center for Population Research, NICHD, Bethesda, 1976.
- Ogbimi AO, Johnson PM. Further immunological and chemical characterisation of human syncytiotrophoblast microvillous membrane-associated components. *J Reprod Immunol* 1981; 2: 99-108.
- Oksenberg JR, Persitz E, Amar A, Brautbar C. Maternal-paternal histocompatibility: lack of association with habitual abortions. *Fertil Steril* 1984; 42: 389-395.
- Olive DL, Franklin RR, Gratkins LV. The association between endometriosis and spontaneous abortion. A retrospective clinical study. *J Reprod Med* 1982; 27: 333-338.
- Ornoy A, Salamon-Arnon J, Ben-Zur Z, Kohn G. Placental findings in spontaneous abortions and stillbirths. *Teratol* 1981; 24: 243-252.
- Ory SJ. Pulsatile luteinising hormone-releasing hormone therapy in women with polycystic ovarian syndrome. *Fertil Steril* 1988; 49: 941-943.
- Overweg J, Engelfriet CP. Cytotoxic leucocyte iso-antibodies formed during the first pregnancy. *Vox Sang* 1969; 16: 97-104.
- Panayotou PP, Kaskarelis DB, Miettinen OS, Trichopoulos DB, Kalandidi AK. Induced abortion and ectopic pregnancy. *Am J Obstet Gynecol* 1972; 114: 507-510.

- Parazzini F, Acaia B, Ricciardiello O, Fedele L, Liati P, Candiani GB. Short-term reproductive prognosis when no cause can be found for recurrent miscarriage. *Br J Obstet Gynaecol* 1988; 95: 654-658.
- Patil SR, Lubs HA. A possible association of long Y chromosomes and fetal loss. *Hum Genet* 1977; 35: 233-235.
- Payne R, Rolfs MR. Feto-maternal leucocyte incompatibility. *J Clin Investigation* 1958; 37: 1756-1763.
- Payne R. The development and persistence of leucoagglutinins in parous women. *Blood* 1962; 19: 411-424.
- Pederson H, Finster M. Anaesthetic risk in the pregnant surgical patient. *Anaesthesiol* 1979; 51: 439-451.
- Pence H, Petty WM, Rocklin RE. Suppression of maternal responsiveness to paternal antigen by maternal plasma. *J Immunol* 1975; 114: 525-528.
- Penrose LS, Delhanty JDA. Triploid cell cultures from a macerated fetus. *Lancet* 1961; i: 1261-1262.
- Pepperell RJ, Evans JH, Brown JB, Smith MA, Healy D, Burger HG. Serum prolactin levels and the value of bromocryptine in the treatment of anovulatory infertility. *Br J Obstet Gynaecol* 1977; 84: 58-66.
- Petersohn L. Fertility in patients with ovarian endometriosis before and after treatment. *Acta Obstet Gynecol Scand* 1970; 49: 331-333.
- Petersson F. Epidemiology of early pregnancy wastage. Stockholm: Svenska Bokförlaget, 1968.
- Petri M, Golbus M, Anderson R, Whiting-O'Keefe Q, Corash L, Hellman D. Anti-nuclear antibody, lupus anticoagulant and anti-cardiolipin antibody in women with idiopathic habitual abortion: a controlled prospective study of forty-four women. *Arthritis Rheum* 1987; 30: 601-606.
- Pharoah POD, Alberman E, Doyle P, Chamberlain G. Outcome of pregnancy among women in anaesthetic practice. *Lancet* 1977; i: 34-36.
- Philip RN, Reinhard KR, Lackman DB. Observations on a mumps epidemic in a "virgin" population. *Am J Epidemiol* 1959; 69: 91-111.
- Phillipe E, Boué JG. La placenta des aberrations chromosomiques létales. *Annales d'Anatomie et Pathologie (Paris)* 1969; 14: 249-266.
- Pike MC, Henderson BE, Casagrande JT. Oral contraceptive use and early abortion as risk factors for breast cancer in young women. *Br J Cancer* 1981; 43: 72-76.
- Plachot M, de Grouchy J, Junca AM, Mandelbaum J, Turleau C, Couillin P, Cohen J, Salt-Baroux J. From oocyte to embryo: a model, deduced from in vitro fertilisation, for natural selection against chromosome abnormalities. *Ann Genet* 1987a; 30: 22-32.
- Plachot M. Chromosome analysis of spontaneous abortions after IVF. A European survey. *Hum Reprod* 1989; 4: 425-429.
- Poland BJ, Miller JR, Jones DC, Trimble BK. Reproductive counselling in patients who have had a spontaneous abortion. *Am J Obstet Gynecol* 1977; 127: 685-691.
- Poliak A, Smith JJ, Romney SL. Clinical evaluation of clomiphene: Clomiphene and human chorionic gonadotrophin: and clomiphene,

- human chorionic gonadotrophin and estrogens in anovulatory cycles. *Fertil Steril* 1973; 24: 921-925.
- Polishuk WZ, Siew FP, Gordon R, Lebenshart P. Vascular changes in traumatic amenorrhoea and hypomenorrhoea. *Int J Fertil* 1977; 22: 189-193.
- Pollack MS, Kirkpatrick D, Kapoor N, Dupont B, O'Reilly RJ. Identification by HLA typing for intrauterine derived maternal T-cells in four patients with severe combined immunodeficiency. *N Eng J Med* 1982; 307: 662-666.
- Polson DW, Wadsworth J, Adams J, Franks S. Polycystic ovaries- a common finding in normal women. *Lancet* 1988; ii: 870-872.
- Porreco RP, Haverkamp AD. Brucellosis in pregnancy. *Obstet Gynecol* 1974; 44: 597-602.
- Power DA, Catto GRD, Mason RJ. The fetus as an allograft: evidence for protective antibodies to HLA-linked paternal antigens. *Lancet* 1983; 2: 701-704.
- Prentice RL, Gatenby PA, Loblay RH, Shearman RP, Kronenberg H, Basten A. Lupus anticoagulant in pregnancy. *Lancet* 1984; ii: 464.
- Prochownik L. *Arch Gynecol* 1877; 11: 403-404.
- Prout TE. Thyroid disease in pregnancy. *Am J Obstet Gynecol* 1975; 122: 669-676.
- Punnönen R, Heinonen PK, Ashorn R, Kujansuu E, Vilja P, Tuohimaa P. Spontaneous luteinising hormone surge and cleavage of in vitro fertilised embryos. *Fertil Steril* 1988; 49: 479-482.
- Purchase IFH, Richardson CR, Anderson D. Chromosomal and dominant lethal effects of vinyl chloride. *Lancet* 1975; ii: 410-411.
- Purdham J. Adverse pregnancy outcome amongst VDU operators - the cluster phenomenon. In: Pearce BG (ed): *Allegations of reproductive hazards from VDUs. Proceedings of an International Scientific Meeting, London, November 1984. Humane Technology*; 27-40.
- Purtilo DT, Hallgren HM, Yunis EJ. Depressed maternal lymphocyte response to phytohaemagglutinin in human pregnancy. *Lancet* 1972; i: 769-771.
- Quigley MM, Berkowitz AS, Gilbert SA, Wolf DP. Clomiphene citrate in an in-vitro fertilisation program: Hormonal comparisons between 50mg and 150mg daily dosages. *Fertil Steril* 1984; 41: 809-815.
- Quinlan RW, Cruz AC. Reproductive failure and cervical incompetence in women exposed to diethylstilboestrol. *S Afr Med J* 1980; 73: 89.
- Quinn PA, Shewchuk AB, Shuber J, Lie KI, Ryan E, Chipman ML, Nocilla DM. Efficacy of antibiotic therapy in preventing spontaneous pregnancy loss among couples colonised with genital mycoplasmas. *Am J Obstet Gynecol* 1983; 145: 239-244.
- Radwanska E, Frankenberg J, Allen EI. Plasma progesterone levels in normal and abnormal early human pregnancy. *Fertil Steril* 1978; 30: 398-402.
- Radwanska E, Hammond J, Smith P. Single midluteal progesterone assay in the management of ovulatory infertility. *J Reprod Med* 1981; 26: 85-89.

- Radwanska E, Swyer GIM. Plasma progesterone estimation in infertile women and in women under treatment with clomiphene and chorionic gonadotrophin. *J Obstet Gynaecol Br Empire* 1974; 81: 107-112.
- Ranney MC. Congenital cervical incompetence in primigravida. *Am J Obstet Gynecol* 1963; 86: 52-65.
- Rappaport F, Rabinovitz M, Toaff R, Krochik N. Genital listeriosis as a cause of repeated abortion. *Lancet* 1960; i: 1273.
- Ratten GJ, Beischer NA. The effect of termination of pregnancy on maturity of subsequent pregnancy. *Med J Aust* 1979; 1: 479-483.
- Redman CWG, Arenas J, Mason D, Sargent IL, Sutton L. Maternal alloimmune recognition of the fetus in human pregnancy. In: Gill TJ, Wegmann TG, Nisbet-Brown E (eds): *Immunoregulation and fetal survival*. New York, Oxford University Press 1987; 210-229.
- Redman CWG, McMichael AJ, Stirrat GM, Sunderland CA, Ting A. Class 1 major histocompatibility antigens on human extravillous trophoblast. *Immunol* 1984; 52: 457-468.
- Reed TE, Kelly EL. The completed reproductive performance of 161 couples selected before marriage and classified by ABO blood group. *Ann Hum Genet* 1958; 22: 165-181.
- Reginald PW, Beard RW, Chapple J, Forbes P B, Liddell H S, Mowbray J F, Underwood JL. Outcome of pregnancies beyond 28 weeks gestation in women with a history of recurrent miscarriage. *Br J Obstet Gynaecol* 1987; 94: 643-648.
- Rehan NE, Sobrero AJ, Fertig JW. The semen of fertile men: statistical analysis of 1300 men. *Fertil Steril* 1975; 26: 492-502.
- Reijnders F, Thomas CMG, Doesburg WH, Rolland J, Eskes TK. Endocrine effects of 17 alpha hydroxyprogesterone caproate during early pregnancy: a double blind clinical trial. *Br J Obstet Gynaecol* 1988; 95: 462-468.
- Ressegue LJ, Hick JF, Bruen JA, Noller KL, O'Fallon WM, Kurland LT. Congenital malformations among offspring exposed in utero to progestins. *Fertil Steril* 1985; 43: 514-519.
- Reyes FI, Koh KS, Faiman C. Fertility in women with gonadal dysgenesis. *Am J Obstet Gynecol* 1976; 126: 668-670.
- Reynolds WA. Thyroid function in mother, fetus and neonate. *Obstet Gynecol Ann* 1978; 7: 67-90.
- Reznikoff-Etievant MF, Durieux I, Huchet J, Salmon C, Netter A. Human MHC antigens and paternal leucocyte injections in recurrent spontaneous abortions. In: Beard RW, Sharp F (eds): *Early pregnancy loss: Mechanisms and treatment*. Springer-Verlag, 1988; 375-384.
- Reznikoff-Etievant MF, Durieux I, Huchet J, Salmon Ch, Netter A. Recurrent spontaneous abortions, HLA antigen sharing and anti-paternal immunity. Immunotherapeutic assay. In: Chaouat G (ed): *Reproductive Immunology: Maternal-Fetal Relationship*. INSERM Publications, Paris 1987; 154: 187-202.

- Reznikoff-Etievant MF, Simonney N, Janaud A, Darbois Y, Netter A. Treatment of recurrent spontaneous abortions by immunisation with paternal leucocytes. *Lancet* 1985; i: 1398.
- Rhoads GG, Jackson LG, Schlesselman SE, De La Cruz FF, Desnick RJ Golbus MS et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. *N Eng J Med* 1989; 320: 609-617.
- Richardson JA, Dixon G. Effects of legal termination on subsequent pregnancy. *Br Med J* 1976; 1: 1303-1304.
- Risch HA, Weiss NS, Clarke AE, Miller A. Risk factors for spontaneous abortion and its recurrence. *Am J Epidemiol* 1988; 128: 420-430.
- Roberts CJ, Lowe CR. Where have all the conceptions gone? *Lancet* 1975; i: 498-499.
- Robertson WB, Brosens I, Landells WN. Abnormal placentation *Obstet Gynecol Ann* 1985; 14: 411-426.
- Rock JA, Wentz AC, Cole KA, Kimball AWJr, Zacur HA, Early SA, Jones GS. Fetal malformations following progesterone therapy during pregnancy: a preliminary report. *Fertil Steril* 1985; 44: 17-19.
- Rock JA, Zacur HA. The clinical management of repeated pregnancy wastage. *Fertil Steril* 1983; 39: 123-140.
- Rocklin RE, Kitzmiller J, Garovoy MR. Maternal-fetal relation. ii. Further characterisation of an immunologic blocking factor that develops during pregnancy. *Clin Immunol Immunopathol* 1982; 22: 305-315.
- Rocklin RE, Kitzmiller JL, Carpenter CB, Garovoy MR, David JR. Maternal-fetal relation. i. Absence of an immunologic blocking factor from the serum of women with chronic abortions. *New Engl J Med* 1976; 295: 375-404.
- Rocklin RE, Kitzmiller JL, Kaye MD. Immunobiology of the maternal fetal relationship. *Ann Rev Med* 1979; 30: 375-404.
- Rocklin RE, Zuckerman JR, Alpert E, David JR. Effect of multiparity on human maternal hypersensitivity to fetal antigen. *Nature* 1973; 241: 130-131.
- Rolfe BE. Detection of fetal wastage. *Fertil Steril* 1982; 37: 655-660.
- Rom WN. Effects of lead on the female and reproduction: a review. *Mount Sinai J Med* 1976; 43: 542-552.
- Roman E, Alberman E, Pharoah POD. Pregnancy order and reproductive loss. *Br Med J* 1980; 1: 715.
- Roman E, Alberman E. Spontaneous abortion, gravidity, pregnancy order and pregnancy interval. In: Porter IH & Hook EB (eds): *Human Embryonic and Fetal Death*. New York: Academic Press, 1980; 129-141
- Roman E, Doyle P, Beral V, Alberman E, Pharoah POD. Fetal loss, gravidity and pregnancy order. *Early Hum Dev* 1978; 2: 131-138.
- Roman E. Fetal loss rates and their relation to pregnancy order. *J Epidemiol Comm Hlth* 1984; 38: 29-35.
- Roman E. Maternal age, pregnancy interval and spontaneous abortion. In: *Spontaneous abortion and its relationship to various maternal and obstetric factors*. PhD Thesis. University of London, 1982.

- Romeu A, Muasher SJ, Acosta AA, Veeck LL, Diaz J, Jones GS, Jones HWJr, Rosenwaks Z. Results of in-vitro fertilisation attempts in women 40 years of age and older: the Norfolk experience. *Fertil Steril* 1987; 47: 130-136.
- Rooney DE, MacLachlan N, Smith J, Rebello MT, Loeffler FE, Beard RW, Rodeck C, Coleman DV. Early amniocentesis: a cytogenetic evaluation. *Br Med J* 1989; 299: 25.
- Ros JO, Tarres MV, Baucells MV, Maird JJ, Solano JT. Prednisone and maternal lupus anticoagulant. *Lancet* 1983; ii: 576.
- Rosenberg SM, Luciano AA, Riddick DH. The luteal phase defect: the relative frequency of, and encouraging response to, treatment with vaginal progesterone. *Fertil Steril* 1980; 34: 17-20.
- Rosenfeld DL, Bronson RA. Reproductive problems in the DES exposed female. *Obstet Gynecol* 1980; 55: 453-456.
- Rosenfeld DL, Garcia CR. A comparison of endometrial histology with simultaneous plasma progesterone determination in infertile women. *Fertil Steril* 1976; 27: 1256-1266.
- Rosenfield A. Oral and intrauterine contraception: a 1978 risk assessment. *Am J Obstet Gynecol* 1978; 132: 92-106.
- Roth DB. The frequency of spontaneous abortion. *Int J Fertil* 1963; 8: 434-437.
- Rothman KJ. Fetal loss, twinning and birth weight after oral contraceptive use. *N Eng J Med* 1977; 297: 468-471.
- Royal College of General Practitioners' Oral Contraceptive Study. The outcome of pregnancy in former oral contraceptive users. *Br J Obstet Gynaecol* 1976; 83: 608-616.
- Royal College of Obstetricians and Gynaecologists. Report on Fetal Viability and Clinical Practice. London: Chameleon Press Ltd, 1985.
- Rubenstein A, Koren Z, Murphy RA. Suppression of maternal lymphocyte mitogenic responses by supernatants from short term placental cell cultures. *Am J Reprod Immunol* 1982; 2: 260-264.
- Rudak E, Dor J, Mashiach S, Nebel L, Goldman B. Chromosome analysis of multipronuclear human oocytes fertilised in vitro. *Fertil Steril* 1984; 4: 538-545.
- Rush D. Examination of the relationship between birthweight, cigarette smoking during pregnancy and maternal weight gain. *J Obstet Gynaecol Br Commonw*; 1974; 81: 746-752.
- Rush RW, Isaacs S, McPherson K, Jones L, Chalmers I, Grant A. A randomised controlled trial of cervical cerclage in women at high risk of spontaneous preterm delivery. *Br J Obstet Gynecol* 1984; 91: 724-730.
- Rushton DI. Examination of products of conception from previable human pregnancies. *J Clin Pathol* 1981; 34: 819-835.
- Rushton DI. Simplified classification of spontaneous abortions. *J Med Genet* 1978; 15: 1-9.
- Rutherford AJ, Subak-Sharpe RJ, Dawson KJ, Margara RA, Franks S, Winston RM. Improvement of in vitro fertilisation after treatment with buserelin, an agonist of luteinising hormone releasing hormone. *Br Med J* 1988; 296: 1765-1768.

- Sachs ES, Jahoda MGJ, Van Hemel JO, Hoozeboom AJM, Sandkuyil LA. Chromosome studies of 500 couples with two or more abortions. *Obstet Gynecol* 1985; 65: 375-378.
- Sagle M, Bishop K, Ridley N, Alexander FM, Michel M, Bonney RC, Beard RW, Franks S. Recurrent early miscarriage and polycystic ovaries. *Br Med J* 1988; 297: 1027-1028.
- Salisbury GW, Hart RG, Lodge JR. The spermatozoan genome and fertility. *Am J Obstet Gynecol* 1977; 128: 342-350.
- Sandberg EC, Riffle NL, Higdon JV, Getman CE. Pregnancy outcome in women exposed to DES in utero. *Am J Obstet Gynecol* 1981; 140: 194-205.
- Sandler B. Anencephaly and ovulation stimulation. *Lancet* 1973; ii: 379.
- Sant-Cassia LJ, Cooke P. Chromosomal analysis of couples with repeated spontaneous abortions. *Br J Obstet Gynaecol* 1981; 88: 52-58.
- Sargent IL, Arenas J, Redman CWG. Maternal cell-mediated sensitisation to paternal HLA may occur, but is not a regular event in normal human pregnancy. *J Reprod Immunol* 1987; 10: 111-120.
- Sargent IL, Wilkins T, Redman CWG. Maternal immune responses to the fetus in early pregnancy and recurrent miscarriage. *Lancet* 1988; ii: 1099-1104.
- Sarram M, Feiz J, Foruzandeh M, Gazantampoun P. Intrauterine fetal infection with *Brucella melitensis* as a possible cause of second trimester abortion. *Am J Obstet Gynecol* 1974; 119: 657-660.
- Sarti DA, Sample WF, Hobel CJ, Staisch KJ. Ultrasonic visualisation of a dilated cervix during pregnancy. *Radiology* 1979; 130: 417-420.
- Saunders P, Milton PJD. Laparotomy during pregnancy: an assessment of diagnostic accuracy and fetal wastage. *Br Med J* 1973; ii: 165-167.
- Schacter B, Muir A, Gyves M, Tasin M. HLA-A, B compatibility in parents of offspring with neural tube defects or couples expressing involuntary fetal wastage. *Lancet* 1979; 1: 796-799.
- Schacter J, Hanner L, Hill EG. Are chlamydial infections the most prevalent venereal disease? *JAMA* 1975; 231: 1252-1255.
- Schenker JG, Margiolath EJ. Intrauterine adhesions: an updated appraisal. *Fertil Steril* 1982; 37: 593-610.
- Schroder J. Transplacental passage of blood cells. *J Med Genet* 1975; 12: 230-242.
- Schwartz RP, Chatwani A, Sullivan P. Cervical cerclage: A review of 74 cases. *J Reprod Med* 1984; 29: 103-106.
- Schwartz S, Palmer CG. Chromosomal findings in 164 couples with repeated spontaneous abortions: with special consideration to prior reproductive history. *Hum Genet* 1983; 63: 28-34.
- Schwartz TF, Roggendorf M, Hottentrager B, Deinhardt F, Enders G, Gloning KP, Schram T, Hansmann M. Human parvovirus B19 infection in pregnancy. *Lancet* 1988; ii: 566-567.
- Schmidt R, Nitowsky HM, Dar H. Cytogenetic studies in reproductive loss. *JAMA* 1976; 236: 369-373.
- Scott JR, Beer AE, Stastny P. Immunogenetic factors in pre-eclampsia and eclampsia: Erythrocyte histocompatibility and Y-dependant antigens. *JAMA* 1976; 235: 402-404.

- Scott JS, Maddison PJ, Taylor PV, Escher E, Scott O, Skinner RP. Connective tissue disease, antibodies to ribonucleoprotein and congenital heart block. *N Eng J Med* 1983; 309: 209-212.
- Scott JS. Systemic lupus erythematosus and allied disorders in pregnancy. *Clin Obstet Gynaecol* 1979; 6: 461-471.
- Seeliger HPR, Emmerling P, Emmerling H. Zur Verbreitung der listeriose in Deutschland. *Dtsch Med Woekenschr* 1968; 93: 2037.
- Seigler HF, Metzgar RS. Embryonic development of human transplantation antigens. *Transpl* 1970; 9: 478-486.
- Sengupta BS, Ramachander N, Zamah N. Salmonella septic abortion. *Int Surg* 1980; 65: 183-186.
- Seppälä M, Vara P. Cervical cerclage in the treatment of incompetent cervix. *Acta Obstet Gynecol Scand* 1970; 49: 343-346.
- Sever J, Terasaki PI. Maternal-fetal incompatibility. III. Central nervous system and cardiac abnormalities. In: WF Bodmer, JR Batchelor JG Bodmer (eds): *Histocompatibility testing* 1970. Munksgaard Copenhagen, 1970; 495-499.
- Shapiro S, Bross D. Risk factors for fetal death in studies of vital statistics data: inference and limitations. In: Porter IH & Hook EB (eds) *Human Embryonic and fetal death*. New York: Academic Press, 1980; 89-105.
- Shapiro S, Levine HS, Abramowicz M. Factors associated with early and late fetal loss. *Adv Plann Parenthood* 1971; 6: 45-58.
- Sharp NC, Anthony F, Miller JF, Masson GM. Early conceptual loss in subfertile patients. *Br J Obstet Gynaecol* 1983; 93: 1072-1081.
- Sharpe RM, Wrixon W, Hobson BM, Corker CS, McLean HA, Short RV. Absence of hCG-like activity in the blood of women fitted with intrauterine contraceptive devices. *J Clin Endocrinol Metab* 1977; 45: 496-499.
- Shearman RP, Garrett WJ. Double blind study of the effect of 17-hydroxyprogesterone caproate on abortion rate. *Br Med J* 1963; 1: 292-295.
- Shepard MK, Senturia YD. Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertil Steril* 1977; 28: 541-548.
- Sher G, Knutzen V, Stratton C, Chotiner H, Mayville J. In vitro fertilisation and embryo transfer: Two year experience. *Obstet Gynecol* 1986; 67: 309-315.
- Shirodkar VN. A new method of operative treatment for habitual abortion in the second trimester of pregnancy. *Antiseptic* 1955; 52: 229-235.
- Short RV. The evolution of human reproduction. *Proc R Soc* 1976; 195: 3-24.
- Siegel M, Fuerst HT, Peress NS. Comparative fetal mortality in maternal virus diseases. A prospective study on rubella, measles, mumps, chickenpox and hepatitis. *N Eng J Med* 1966; 274: 768-771.
- Siiteri PK, Stites DP. Immunologic and endocrine interrelationships in pregnancy. *Biol Reprod* 1982; 26: 1-14.
- Simoni G, Gimelli G, Cuoco C. Discordance between prenatal cytogenetic diagnosis after chorionic villus sampling and chromosomal

- constitution of the fetus. In: Fraccaro G, Simoni G, Brambati B (eds). First trimester Fetal Diagnosis. Berlin, Springer-Verlag, 1985, 137-143.
- Simor AE, Karmali MA, Jadavji T, Roscoe M. Abortion and perinatal sepsis associated with campylobacter infection. *Rev Infect Dis* 1986; 8: 397-402.
- Simpson ER, MacDonald PC. Endocrinology of pregnancy. In: Williams RH (ed) Textbook of Endocrinology 6th ed. Philadelphia, WB Saunders, 1981: 412-422.
- Simpson JL, Elias S, Martin AO. Parental chromosomal rearrangements associated with repetitive spontaneous abortions. *Fertil Steril* 1981; 36: 584-590.
- Simpson JL, Bombard A. Chromosomal abnormalities in spontaneous abortion: Frequency, pathology and genetic counselling. In: Bennett MJ, Edmonds DK (eds). Spontaneous and recurrent abortion. Oxford: Blackwell Scientific Publications, 1987; 51-76.
- Simpson JL. Genes, chromosomes and reproductive failure. *Fertil Steril* 1980; 33: 107-116.
- Simpson JL. Low fetal loss rate after normal ultrasound at eight weeks gestation: implications for chorionic villus sampling (CVS). The Diabetes in Early Pregnancy Project NICHD. *Am J Hum Genet* 1984; 36: 197 (suppl).
- Simpson JL. Repetitive spontaneous abortion. In: Porter IH (ed): Perinatal Genetics. New York: Academic Press, 1986.
- Simpson NE, Dallaire L, Miller JR, Siminovich L, Hamerton JL, Miller J, McKen C. Prenatal diagnosis of genetic disease in Canada: report of a collaborative study. *Can Med Ass J* 1976; 115: 739-746.
- Singal DP, Butler L, Liao SK, Joseph S. The fetus as an allograft: Evidence for anti-idiotypic antibodies induced by pregnancy. *Am J Reprod Immunol* 1984; 6: 145-151.
- Singer MS, Hochman M. Incompetent cervix in hormone exposed offspring. *Obstet Gynecol* 1978; 51: 625-626.
- Singh DN, Hara S, Foster HW, Grimes EM. Reproductive performance in women with sex chromosome mosaicism. *Obstet Gynecol* 1980; 55: 608-611.
- Singh RP, Carr DH. Anatomic findings in human abortions of known chromosomal constitution. *Obstet Gynecol* 1967; 29: 806-818.
- Smart YC, Cripps AW, Clancy RL, Roberts TK, Lopata A, Shutt DA. Detection of an immunosuppressive factor in human preimplantation embryo cultures. *Med J Aust* 1981; 1: 78-79.
- Smidt-Jensen S, Hahnemann N. Transabdominal fine needle biopsy from chorionic villi in the first trimester. *Prenat Diagn* 1984; 4: 163-169.
- Smidt-Jensen S, Philip J. Transabdominal CVS versus transcervical versus amniocentesis: a randomised study. *Am J Hum Genet* 1987; 41: 27 (Abstract).
- Smith AH, Fisher DO, Pearce N, Chapman CJ. Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. *Arch Env Health* 1982; 37: 197-200.

- Smith JB, Cowchock FS. Immunological studies in recurrent spontaneous abortion: effects of immunisation of women with paternal mononuclear cells. *J Reprod Immunol* 1988; 14: 99-113.
- Smith OW. Diethylstilboestrol in the prevention and treatment of complications of pregnancy. *Am J Obstet Gynecol* 1948; 56: 821-826.
- Smith SK, Lenton EA, Landgren E, Cooke ID. The short luteal phase and infertility. *Br J Obstet Gynaecol* 1984; 91: 1120-1122.
- Sobowale O, Lenton EA, Francis B, Cooke ID. Comparison of plasma steroid and gonadotrophin profiles in spontaneous cycles in which conception did and did not occur. *Br J Obstet Gynaecol* 1978; 85: 460-467.
- Sokol RJ, Miller SI, Reed G. Alcohol abuse during pregnancy: an epidemiologic study. *Clin Exp Res* 1980; 4: 135-145.
- Sompolinsky D, Solomon F, Elkina L, Weinraub Z, Bukovsky I, Caspi E. Infections with Mycoplasma and bacteria in induced midtrimester abortion and fetal loss. *Am J Obstet Gynecol* 1975; 121: 610-616.
- Soulie JP, Boffa MC. Avortements a répétition, thromboses et anticoagulant circulant anti-thromboplastine. *Nouv Presse Med* 1980; 9: 859-864.
- Southern PMJr. Habitual abortion and toxoplasmosis. *Obstet Gynecol* 1972; 39: 45-47.
- Spark RF, Dickstein G. Bromocryptine and endocrine disorders. *Ann Intern Med* 1979; 90: 949-956.
- Speert H. Pregnancy prognosis following repeated abortion. *Am J Obstet Gynecol* 1954; 68: 665-673.
- Speidel BD, Meadow SR. Maternal epilepsy and abnormalities of the fetus. *Lancet* 1972; ii: 839.
- Spence AA. Occupational hazards in the United Kingdom. *Int Anaesth Clin* 1981; 19: 165-176.
- Spencer JW, Cox DN. Emotional responses of pregnant women to chorionic villi sampling or amniocentesis. *Am J Obstet Gynecol* 1987; 157: 1155-1160.
- Spinks NR, O'Neill C. Embryo-derived platelet-activating factor is essential for establishment of pregnancy in the mouse. *Lancet* 1987; i: 106-107.
- Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Walton PD, Veren DA, Page F, Alford CA. Primary cytomegalovirus infection in pregnancy: Incidence, transmission to fetus, and pregnancy outcome. *JAMA* 1986; 256: 1904-1908.
- Stagno S, Pass RF, Dworsky ME, Henderson RE, Moore EG, Walton PD, Alford CA. Congenital cytomegalovirus infection: The relative importance of primary and recurrent maternal infection. *N Eng J Med* 1982; 306: 945-949.
- Stanger JD, Yovich JL. Reduced in-vitro fertilisation of human oocytes from patients with raised basal LH levels during the follicular phase. *Br J Obstet Gynaecol* 1985; 92: 385-393.
- Stastny P. Tissue typing antisera from immunization by pregnancy. *Tissue Antigens* 1972; 2: 123-127.
- Steele MW, Breg WT. Chromosome analysis of human amniotic fluid cells. *Lancet* 1966; i: 383.

- Stein Z, Kline J, Susser E, Shrout P, Warburton D, Susser M. Maternal age and spontaneous abortion. In: Porter IH & Hook EB (eds): Human Embryonic and Fetal Death. New York: Academic Press, 1980; 107-127
- Stein Z, Susser M, Warburton D, Wittes J, Kline J. Spontaneous abortion as a screening device: the effect of fetal survival on the incidence of birth defects. *Am J Epidemiol* 1975; 102: 275-290.
- Stenchever MA, Parks KA, Daines TL, Allen MA, Stenchever MR. Cytogenetics of habitual abortion and other reproductive wastage. *Am J Obstet Gynecol* 1977; 127: 143-150.
- Stenchever MA. Managing habitual abortion. *Cont Obstet Gynecol* 1980; 16: 23-24.
- Stene J, Stene E, Mikkelsen M. Risk for chromosomal abnormality at amniocentesis following a child with a non-inherited chromosome aberration. *Prenat Diagn* 1984; 4: 81-95.
- Stevenson AC, McClarin RH. Determination of the sex of human abortions by nuclear sexing the cells of the chorionic villi. *Nature* 1957; 180: 198-199.
- Stevenson AC, Warnock NA. Observations on the results of pregnancies in women resident in Belfast. I. data relating to all pregnancies ending in 1957. *Ann Hum Genet* 1959; 23: 382-414.
- Stimson WH, Strachan AF, Shepherd A. Studies on the maternal immune response to placental antigens: absence of a blocking factor from the blood of abortion prone women. *Br J Obstet Gynaecol* 1979; 86: 41-45.
- Stites DP, Pavia CS, Clemens LE, Kuhn RW, Siiteri PK. Immunologic regulation in pregnancy. *Arthritis Rheum* 1979; 22: 1300-1307.
- Stoot JEGM. Aangeboren afwijkingen van de uterus en gestoorde voortplanting. PhD Thesis 1978. University of Nijmegen, Holland.
- Stouffer RL. Perspectives on the corpus luteum of the menstrual cycle and early pregnancy. *Semin Reprod Endocrinol* 1988; 6: 103-113.
- Strandberg M, Sandbäck K, Axelson O, Sundell L. Spontaneous abortions among women in hospital laboratory. *Lancet* 1978; i: 384-385.
- Strassman EO. Fertility and unification of double uterus. *Fertil Steril* 1966; 17: 165-176.
- Stray-Pedersen B, Eng J, Reikvam TM. Uterine T-mycoplasma colonisation in reproductive failure. *Am J Obstet Gynecol* 1978; 130: 307-311.
- Stray-Pedersen B, Lorentzen-Styr A. Uterine toxoplasma infections and repeated abortions. *Am J Obstet Gynecol* 1977; 128: 716-721.
- Stray-Pedersen B, Stray-Pedersen S. Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *Am J Obstet Gynecol* 1984; 148: 140-146.
- Stray-Pedersen B. A prospective study of acquired toxoplasmosis among 8,043 pregnant women in the Oslo area. *Am J Obstet Gynecol* 1980; 136: 399-406.
- Strobino B, Fox HE, Kline J, Stein Z, Susser M, Warburton D. Characteristics of women with recurrent spontaneous abortions and women with favourable reproductive histories. *Am J Publ Hlth* 1986; 76: 986-991.
- Strobino B, Kline J, Stein Z. Chemical and physical exposures: Effects on human reproduction and offspring. *Early Hum Dev* 1978; 1: 371-385.

- Stromme WB, Haywa EW. Intrauterine fetal death in the second trimester. *Am J Obstet Gynecol* 1963; 85: 223-233.
- Strott CA, Cargille CM, Ross GT, Lipsett MB. The short luteal phase. *J Clin Endocrinol Metab* 1970; 30: 246-251.
- Sucia-Foca N, Reed E, Rohowsky C, Kung F, King DW. Anti-idiotypic antibodies to anti-HLA receptors induced by pregnancy. *Proc Natl Acad Sci USA* 1983; 80: 830-834.
- Sunderland CA, Redman CWG, Stirrat GM. HLA A,B,C antigens are expressed on nonvillous trophoblast of the early human placenta. *J Immunol* 1981; 127: 2614-2615.
- Sutton L, Mason DY, Redman CWG. HLA-DR positive cells in the human placenta. *Immunol* 1983; 49: 103-112.
- Szulman AE. ABO incompatibility in foetal wastage. *Res Reprod* 1973; 5: 3-4.
- Tabor A, Masden M, Obel EB, Philip J, Bang J, Norgaard-Peterson B. Randomised controlled trial of genetic amniocentesis in 4606 low risk women. *Lancet* 1986; ii: 1287-1293.
- Takahara H, Ohama K, Fujiwara A. Cytogenetic study in early spontaneous abortion. *Hiroshima J Med Sci* 1977; 26: 291-296.
- Takakuwa K, Kanazawa K, Takeuchi S. Production of blocking antibodies by vaccination with husband's lymphocytes in unexplained recurrent aborters: The role in successful pregnancy. *Am J Reprod Immunol Microbiol* 1986; 10: 1-9.
- Takano K, Miller JR. ABO-incompatibility as a cause of spontaneous abortion: evidence from abortuses. *J Med Genet* 1972; 9: 144-150.
- Takeuchi S. Immunology of spontaneous abortion and hydatidiform mole. *Am J Reprod Immunol* 1980; 1: 23-28.
- Tamaya T, Nakata Y, Ohno Y, Nioka S, Furata M, Okada H. The mechanism of action of the copper IUCD. *Fertil Steril* 1976; 27: 767-772.
- Tannenbaum TN, Goldberg RJ. Exposure to anaesthetic gases and reproductive outcome. A review of epidemiologic literature. *J Occup Med* 1985; 27: 659-668.
- Tatum HJ, Schmidt FH, Jain AK. Management and outcome of pregnancies associated with the Copper T intrauterine contraceptive device. *Am J Obstet Gynecol* 1976; 126: 869-879.
- Taylor C, Faulk WP, McIntyre JA. Prevention of recurrent spontaneous abortions by leucocyte transfusions. *J R Soc Med* 1985; 78: 623-627.
- Taylor CG, Faulk WP. Prevention of recurrent abortion with leucocyte transfusions. *Lancet* 1981; ii: 68-70.
- Taylor-Robinson W, McCormack WM. The genital mycoplasmas. *N Eng J Med* 1980; 302: 1003-1010.
- Taymor ML. Use and abuse of clomiphene citrate. *Fertil Steril* 1986; 47: 206-207.
- Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. *Nature* 1964; 204: 998-1000.
- Terasaki PI, Mickey MR, Yamazaki JN, Vredevoe D. Maternal-fetal incompatibility. I. Incidence of HLA antibodies and possible association with congenital anomalies. *Transplantation* 1970; 9: 538-543.

- Thiede HA, Metcalfe S. Chromosomes and human pregnancy wastage. *Am J Obstet Gynecol* 1966; 96: 1132-1138.
- Thlikainen A, Schroder J, De La Chapelle A. Fetal leucocytes in the maternal circulation after delivery. II. Masking of HLA antigens. *Transplantation* 1974; 17: 355-360.
- Tho PT, Byrd JR, McDonough PG. Etiologies and subsequent reproductive performance of 100 couples with recurrent abortion. *Fertil Steril* 1979; 32: 389-395.
- Thomas ML, Harger JH, Wagener DK, Rabin BS, Gill TJ. HLA sharing and spontaneous abortion in humans. *Am J Obstet Gynecol* 1985; 151: 1053-1061.
- Thompson S, Lopez B, Wong K-H. A prospective study of chlamydia and mycoplasma infections during pregnancy: relation to pregnancy outcome and maternal morbidity. In: Mardh PA, Holmes KK, Oriel JD (eds): *Chlamydial infections*. Amsterdam: Elsevier Biomedical, 1983.
- Thorner MO, Edwards CRW, Charlesworth M, Dacie JE, Moulton PJA, Rees LH, Jones AE, Besser GM. Pregnancy in patients presenting with hyperprolactinaemia. *Br Med J* 1979; 2: 771-774.
- Tibiletti MG, Simoni G, Terzoli GL, Romitti L, Fedele L, Candiani GB. Pericentric inversion of chromosome 9 in couples with repeated spontaneous abortion. *Acta Eur Fert* 1981; 12: 245-248.
- Tietze C, Guttmacher AF, Rubin S. Unintentional abortion in 1,497 planned pregnancies. *JAMA* 1950; 142: 1348-1350.
- Tietze C, Martin CE. Fetal deaths, spontaneous and induced, in the urban white population of the United States. *Popul Stud (NY)* 1957; 11: 170-176.
- Tincani A, Cattaneo M, Martinelli M, Faden D, Tarantini M, Cerni V, Balestrieri G. Anti-phospholipid antibodies in recurrent fetal loss: one side of the coin? *Clin Exp Rheumatol* 1987; 4: 390-392..
- Toaff R, Ballas S. Traumatic hypomenorrhoea-amenorrhoea (Asherman's syndrome). *Fertil Steril* 1978; 30: 379-387.
- Toaff R, Toaff ME, Ballas S, Ophir A. Cervical incompetence: diagnostic and therapeutic aspects. *Isr J Med Sci* 1977; 13: 39-49.
- Tompkins P. Comments on the bicornuate uterus and twinning. *Surg Clin North Am* 1962; 42: 1049-1053.
- Tongio MM, Berrebi A, Mayer S. A study of lymphocytotoxic antibodies in multiparous women having had at least four pregnancies. *Tissue Antigens* 1972; 2: 378-388.
- Tongio MM, Mayer S. Narrowing of fetomaternal immunisation at time of delivery. *Tissue Antigens* 1977; 9: 174-176.
- Tsafriri A, Pomerantz SH. Oocyte maturation inhibitor. *Clin Endocrinol Metab* 1986; 15: 157-170.
- Tulchinski D, Hobel CJ. Plasma human chorionic gonadotrophin, estrone, estradiol, estriol, progesterone and 17 alpha-hydroxyprogesterone in human pregnancy. *Am J Obstet Gynecol* 1973; 117: 884-893.
- Tupper C, Moya F, Stewart LC, Weil RJ, Gray JD. The problem of spontaneous abortion. *Am J Obstet Gynecol* 1957; 73: 313-321.

- Turleau C, Chavin-Colin F, DeGrouchy J. Cytogenetic investigation in 413 couples with spontaneous abortions. *Eur J Obstet Gynaecol Reprod Biol* 1980; 9: 65-74.
- Turnbull AC, Mackenzie IZ. Second trimester amniocentesis and termination of pregnancy. *Brit Med Bull* 1983; 39: 315-321.
- Tyrey L, Hammond CB. The HCG Beta subunit radioimmunoassay: potential error in HCG measurement related to choice of labelled antigen. *Am J Obstet Gynaecol* 1976; 125: 160-165.
- Uchida IA, Holunga R, Lawler C. Maternal radiation and chromosomal aberration. *Lancet* 1968; ii: 1045-1049.
- Unander AM, Lindholm A, Olding LB. Blood transfusions generate/increase previously absent/weak blocking antibody in women with habitual abortion. *Fertil Steril* 1985; 44: 766-771.
- Unander AM, Lindholm A. Transfusions of leucocyte-rich erythrocyte concentrates: A successful treatment in selected cases of habitual abortion. *Am J Obstet Gynecol* 1986; 154: 516-520.
- Unander AM, Olding LB. Habitual abortion: parental sharing of HLA antigens, absence of maternal blocking antibody and suppression of maternal lymphocytes. *Am J Reprod Immunol* 1983; 4: 171-178.
- Unander AM, Olding LB. Ontogeny and postnatal persistence of a strong suppressor activity in man. *J Immunol* 1981; 127: 1182-1186.
- Underwood JL, Ruszkiewicz M, Barnden KL, Beard RW, Mowbray JF, Sanderson AR. Does antigenic modulation cause the absence of major histocompatibility complex antigens on the syncytiotrophoblast. *Transpl Proc* 1985; 17: 921-934.
- Vaisman AI. Work in operating theatres and its effect on the health of anaesthesiologists. *Eksp Khir Anestisiol* 1967; 12: 44-49.
- Van Blerkom J, Henry G, Porreco R. Preimplantation human embryonic development from polypronuclear eggs after in vitro fertilisation. *Fertil Steril* 1984; 41: 686-696.
- Van Rood JJ, Van Leeuwen A, Eernisse JG. Leucocyte antibodies in sera from pregnant women. *Nature* 1958; 181: 1735-1736.
- Vere MF. A ten year survey of the use of cervical cerclage. *Br J Clin Practice* 1982; 36: 302-310.
- Veridiano NP, Delke I, Rogers J, Tancer ML. Reproductive performance of DES exposed female progeny. *Obstet Gynecol* 1981; 58: 58-61.
- Vermeylen J, Blockmans D, Spitz B. Thrombosis and immune disorders. *Clin Haematol* 1986; 15: 1-20.
- Verp MS, Rzeszutarski MS, Martin AO, Simpson JL. Relationship between Y chromosome length and first trimester spontaneous abortion. *Am J Obstet Gynecol* 1983; 145: 433-438.
- Verp MS, Simpson JL. Prenatal diagnoses of cytogenetic abnormalities. In: Filkins K, Kaminetsky H (eds): *Prenatal Diagnosis*. New York: Dakker, 1985.
- Vessey M, Doll R, Petro R, Johnson R, Wiggins P. A long term follow up study of women using different methods of contraception: an interim report. *J Biosoc Sci* 1976; 8: 373-427.

- Vessey MP, Johnson D, Doll R, Peto R. Outcome of pregnancy in women using an intrauterine device. *Lancet* 1974; i: 495-499.
- Vessey MP, Wright NH, McPherson K, Wiggins P. Fertility after stopping different methods of contraception. *Br Med J* 1978; 1: 265-267.
- Vives J, Gelabert A, Castillo R. HLA antibodies and period of gestation: Decline in frequency of positive sera during last trimester. *Tissue Antigens* 1976; 7: 209-212.
- Von Fliedner VE, Merica H, Jeannet M, Barras C, Feldges A, Imbach P, Wyss M. Evidence for HLA-linked susceptibility factors in childhood leukaemia. *Hum Immunol* 1983; 8: 183-193.
- Walker EM, Lewis M, Cooper W, Marnie M and Howie PW. Occult biochemical pregnancy: fact or fiction? *Br J Obstet Gynaecol* 1988; 95: 659-663.
- Walshe JM. Pregnancy in Wilson's disease. *Quart J Med* 1977; 46: 73-83.
- Warburton D, Fraser FC. On the probability that a woman who has had a spontaneous abortion will abort in subsequent pregnancies. *J Obstet Gynaecol Br Commonw* 1961; 68: 784-788.
- Warburton D, Fraser FC. Spontaneous abortion risks in man: data from reproductive histories collected in a medical genetics unit. *Hum Genet* 1964; 16: 1-25.
- Warburton D, Hutzler M, Kline J, Stein Z, Strobino B. Recurrence risk for trisomy following a trisomic spontaneous abortion. American Society of Human Genetics Annual Meeting 1982, Detroit, Michigan.
- Warburton D, Kline J, Stein Z, Susser M. Monosomy X: a chromosomal anomaly associated with young maternal age. *Lancet* 1980b; i: 167-169.
- Warburton D, Stein Z, Kline J, Susser M. Chromosome abnormalities in spontaneous abortion: data from the New York City study. In: Porter IH & Hook EB (eds): *Human Embryonic and Fetal Death*. New York: Academic Press, 1980a; 261-287.
- Warburton D, Susser M, Stein Z, Kline J. Genetic and epidemiological investigation of spontaneous abortion: relevance to clinical practice. *Birth Defects* 1979; 15: 127-136.
- Ward BE, Henry GP, Robinson A. Cytogenetic studies in 100 couples with recurrent spontaneous abortions. *Am J Hum Genet* 1980; 32: 549-554.
- Warren CW, Gold J, Tyler CW, Smith JC, Paris AL. Seasonal variations in spontaneous abortions. *Am J Pub Hlth* 1980; 70: 1297-1299.
- Waterson AP. Virus infections (other than rubella) during pregnancy. *Br Med J* 1979; 2: 564-566.
- Watts DH, Eschenbach DA. Reproductive tract infections as a cause of abortion and preterm birth. *Semin Reprod Endocrinol* 1988; 6: 203-215.
- Weekes ARL, Atlay RD, Brown VA, Jordan EC, Murray SM. The retroverted gravid uterus and its effect on the outcome of pregnancy. *Br Med J* 1976; 1: 622-624.
- Weiland HT, Vermey-Keers C, Salimans MM, Fleuren GJ, Verwey RA, Anderson MJ. Parvovirus B19 associated with fetal abnormality. *Lancet* 1987; i: 682.

- Weir WC, Hendricks CH. The reproductive capacity of an infertile population. *Fertil Steril* 1969; 20: 289-298.
- Weiss DB, Levene C, Aboulafia Y, Isacsohn M. Anti-PP₁P^k (anti-Tj^a) and habitual abortion. *Fertil Steril* 1975; 26: 901-904.
- Weitkamp LR, VanRood JJ, Thorsby E, Bias W, Fotino M, Lawler SD, Dausset J, Mayr WR, Bodmer J. The relation of parental sex and age to recombination in the HLA system. *Hum Hered* 1973; 23: 197-213.
- Wells M, Hsi BL, Faulk WP. Class 1 antigens of the major histocompatibility complex on cytotrophoblast of the human placental basal plate. *Am J Reprod Immunol* 1984; 6: 167-174.
- Wennergren M, Karlsson K, Olsson T. A scoring system for antenatal identification of fetal growth retardation. *Br J Obstet Gynaecol* 1982; 89: 520-524.
- Wentz AC, Herbert CM, Maxson WS, Garner CH. Outcome of progesterone treatment of luteal phase inadequacy. *Fertil Steril* 1984; 41: 856-862.
- Wentz AC, Herbert CM, Maxson WS, Hill GA, Pittaway DE. Cycle of conception endometrial biopsy. *Fertil Steril* 1986; 46: 196-199.
- Wentz AC. Diagnosing luteal phase inadequacy. *Fertil Steril* 1982; 37: 334-335.
- Wentz AC. Endometrial biopsy in the evaluation of infertility. *Fertil Steril* 1980; 33: 121-124.
- Wentz AC. Physiologic and clinical considerations in luteal phase defects. *Clin Obstet Gynecol* 1979; 22: 169-185.
- Weseley AC. Habitual abortion. Two factors of increasing frequency. *Diagn Gynecol Obstet* 1981; 3: 119-121.
- Westerholme P, Ericson A. Pregnancy outcome and VDU work in a cohort of insurance clerks. *Proceedings of an International Scientific Conference, Stockholm* 1986; 665-668.
- Wheeler JM, Johnston BM, Malinak LR. The relationship of endometriosis to spontaneous abortion. *Fertil Steril* 1983; 39: 656-660.
- Whitehouse B. Discussion on the early causes of abortion and sterility. *Proc R Soc Med* 1930; 23: 237-253.
- Whitfield CR, Smith NC, Cockburn F, Gibson AAM. Perinatally related wastage - a proposed classification of primary obstetric factors. *Br J Obstet Gynaecol* 1986; 93: 694-703.
- Whittaker PG, Taylor A, Lind T. Unsuspected pregnancy loss in healthy women. *Lancet* 1983; i: 1126-1127.
- WHO Recommended definitions, terminology and format for statistical tables related to the perinatal period. *Acta Obstet Gynaecol Scand* 1977; 56: 247-253.
- WHO. Spontaneous and induced abortion. Technical report series. No. 461, 1970.
- Whyte A, Loke YW. Antigens of the human trophoblast membrane. *Clin Exp Immunol* 1979; 37: 359-366.
- Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer MS, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. *N Eng J Med* 1988; 319: 189-194.

- Wilson RD, Kendrick V, Wittman BK, McGillivray BC. Risk of spontaneous abortion in ultrasonically normal pregnancies. *Lancet* 1984; ii: 920-921.
- Wimmers MSE, Van der Merwe JV. Chromosome studies on early human embryos fertilised in vitro. *Hum Reprod* 1988; 2: 137-142.
- Winer-Sorgen S, Brown J, Ono T, Gale JA, Campeau JD, Marrs RP, DiZerega GS. Oocyte maturation inhibitor activity in human follicular fluid: quantitative determination in unstimulated and clomiphene citrate - and human menopausal gonadotrophin - stimulated ovarian cycles. *J In Vitro Fertil Embryo Trans* 1986; 3: 218-223.
- Wing ES, Troppoli DV. The intrauterine transmission of typhoid. *JAMA* 1930; 95: 405-406.
- Winikoff D, Malinek M. The predictive value of thyroid test profile in habitual abortion. *Br J Obstet Gynaecol* 1975; 82: 760-766.
- Witkin SS, Davis SS. Effect of sperm antibodies on pregnancy outcome in a subfertile population. *Am J Obstet Gynecol* 1988; 158: 59-62.
- Woernle CH, Anderson LJ, Tattersall P, Davison JM. Human parvovirus B19 infection during pregnancy. *J Infect Dis* 1987; 156: 17-20.
- Woodrow JC, Finn R. Transplacental haemorrhage. *Br J Haematol* 1966; 12: 297-309.
- Wramsby H, Fredga K, Liedholm P. Chromosome analysis of human oocytes recovered from preovulatory follicles in stimulated cycles. *N Eng J Med* 1987; 316: 121-124.
- Wright AD, Nicholson HO, Pollock A, Taylor KG, Betts S. Spontaneous abortion and diabetes mellitus. *Postgrad Med J* 1983; 59: 295-304.
- Wright CSW, Campbell S, Beazley J. Second trimester abortion after vaginal termination of pregnancy. *Lancet* 1972; i: 1278.
- Yen SSC. Chronic anovulation caused by peripheral endocrine disorders. In: Yen SSC, Jaffe R (eds): *Reproductive endocrinology*, 2nd edition. Philadelphia: WB Saunders Co., 1986, 441-499.
- Yerushalmy J, Bierman JM, Kemp DH, Connor A, French FE. Longitudinal studies of pregnancy on the island of Kauai, Territory of Hawaii. *Am J Obstet Gynecol* 1956; 71: 80-86.
- Yip SK, Sung ML. Plasma progesterone in women with a history of recurrent early abortion. *Fertil Steril* 1977; 28: 151-155.
- Young EJ. Human brucellosis. *Rev Infect Dis* 1983; 5: 821-840.
- Young SR, Wade RV, Watt GW, Hixson ET, Dennis EJ. The results of 1000 consecutive prenatal diagnoses. *Am J Obstet Gynecol* 1983; 147: 181-188.
- Youngs ER, Roberts C. *Campylobacter* carriage and pregnancy. *Br J Obstet Gynaecol* 1985; 92: 541-542.
- Youtananukorn V, Matangasombut P, Osathanondh V. Onset of human cell-mediated immune reaction to placental antigens during the first pregnancy. *Clin Exp Immunol* 1974; 16: 593-598.
- Yovich JL, Matson PL. Early pregnancy wastage after gamete manipulation. *Br J Obstet Gynaecol* 1988; 95: 1120-1127.
- Yovich JL, Matson PL. Pregnancy wastage following IVF and related techniques. In: *Proceedings of the international symposium on In-Vitro Fertilisation and Embryo Transfer*, Dubrovnik, October 1987.

Zacur HA, Mitch WE. Renal disease in pregnancy. *Med Clin North Am* 1977; 61: 89-91.

Zipursky A, Pollock J, Neelands P, Chown B, Israels LG. The transplacental passage of foetal red blood cells and the pathogenesis of Rh immunisation during pregnancy. *Lancet* 1963; ii: 489-493.

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