INTRODUCTION

The human foetus is a semiallograft in the maternal host having inherited half of its genotypes from the father. The mechanisms preventing the immune rejection of the foetus are numerous and not yet clearly established. One popular hypothesis is that the foetomaternal disparity among the HLA antigens may actually confer some means of protection (by inducing specific suppression of the maternal immune response) on the developing conceptus. The putative necessity of a sufficient antigenic stimulation of the mother by the foetus for a successful pregnancy, and the converse view that an inadequate maternal immune response to foetal antigens in spontaneous abortion have been suggested by many workers (Gill, 1983 ; Mettler & Paul, 1984; Mowbray & Underwood, 1985).

The marked polymorphism of molecules encoded within the major histocompatibility complex (MHC) ensures that out-bred pregnancy involves materno-foetal genetic disparity. Since, MHC antigens are the focus of mechanisms of T-cell restriction, rejection of allografts and control of the immune response, they are presumed to be of importance in the immunogenetic enigma of successful viviparity (Beer & Billingham, 1977 ; Johnson *et al.*, 1988). Although foetal trophoblast does not normally express classical

HLA antigens at foeto-maternal interfaces (Bulmer & Johnson, 1985), extravillous cytotrophoblast populations do express a 40 kD class-I like MHC antigen linked to β_2-microglobulin (Ellis et al.,1986 ; Johnson & Stern, 1986). In addition, alloantibodies have been demonstrated in some multiparous sera which identify class I - like MHC molecules and also analysis of placentallyeluted antibody has shown frequent reactivity against HLA like determinants other than classical HLA alloantigens (Fauchet et al., 1986 ; Gazit et al., 1984 ; Van Leeuwen et al., 1985 ; Konaeda et al., 1986). Pregnancy induced responses to foetal alloantigens may generate host responses which are more elusive to identify than, for example, cytotoxic responses to classical HLA antigens.

Since the discovery of the HLA antigens in the late fifties and early sixties, the MHC has become one of the most widely studied regions of the human genome. The initial interest in MHC arose from its application in donor selection during organ transplantation (Dausset, 1981). Later, with the demonstration of the existance of specific immune response (Ir) genes within this complex and their involvement in T cell activation and immunoregulatory mechanisms of the body, a new dimension has been added to the understanding of the mechanism of disease susceptibility in man (Van Rood, 1981).

The human major histocompability complex (MHC) consists

of co-dominant genes, encompassing a 2cM(Centimorgan) region on the short-arm of chromosome 6 at $6p^{21.1}$ to $6p^{21.3}$ and is about 4000 Kb in size representing about 2.5% of the entire length of the chromosomes. The MHC consists of a large family of closely related genes sub-divided in groups and referred to as, class-I (HLA-A, -B and -C), class-II (HLA-DR, -DQ, -DC and -SB) and class III (Complement factor) genes. MHC antigens inherited from mother and father are co-dominantly expressed on the cell surface. Closely linked HLA antigens are usually inherited en-block to the next generation, known as a haplotype.

The presence of anti-HLA antibodies in mother against the foetus has drawn considerable attention of the immunogeneticists from several points of view : i) the nature and type of specificity of the lymphocytotoxins present during pregnancy and their relationship to bad obstetric history of the patient, (ii) Possible association of HLA antigens, if there is any, with the unsuccessful pregnancies, (iii) the influence of HLA compatibility between the patient and her spouse on the final outcome of the pregnancy.

These antigens can cross the placental barrier and cause maternal sensitization leading to the formation of alloantibodies. The existance of HLA alloantibodies in the sera of pregnant women were observed by several workers (Payne & Rolfs, 1958 ; Van Rood et al., 1958 ; Terasaki et al., 1970 ; Tongio et al., 1972 ; Vives et al., 1976 ; Gelabert et al., 1981 ; Unander 1983). It has

been reported that the incidence of these antibodies bearing 10% in primiparous women and upto 40% in multiparous women (Terasaki et al., 1970). A foetus exhibits the characteristic of a semiallogeneic graft. This implies that there could be a corresponding maternal immune response to the foetus in the evolution of normal pregnancy. This immune response possibly have some positive consequences rather than negative ones, as it seems to prevent repeated miscarriages (Rocklin et al., 1973 ; Dumble et al., 1977 ; Linander & Odling, 1983).

HLA typing of couples in cases of habitual abortions does not reveal any significant departure (Rocklin et al., 1976; Lauritsen et al., 1976). Beer et. al., (1981) have observed that women with recurrent consecutive spontaneous abortions of known etiology had significantly increased homozygosity between spouses is associated with the post fertilization pregnancy wastage in humans. The entire HLA compatibility between mates also lead to repeated abortion and this is suggested to be due to recessive Ir genes (Redman, 1978). A higher incidence of congenital anomalies have been reported amongst infants of anti-HLA antibody positive mothers (Tarasaki et al., 1970). These antibodies may therefore exert a deletorious effect on the foetus in subsequent pregnancies.

Some studies have pointed towards the increased parental HLA sharing than would be expected by chance (Thomas et al., 1985; MeIntyre et al., 1986), although this does not achieved

statistical significance at all centres (Oksenbegr et al., 1984 ; Johnson et al., 1985).

Several workers (Tongio et al., 1972 ; Vives et al., 1976 ; Gelabert et al., 1981) have shown that in normal pregnant women, these antibodies may be detected after single pregnancy and with successive pregnancies the antibodies are more marked. Vives et al., (1976) have shown that these antibodies are boosted in the first trimester and falls towards term. It rises again to a peak in the immediate post-natal period and falling again with time. It has been shown that the cytotoxic effect of maternal lymphocytes on cultured throphoblast was completely prevented by the presence of maternal serum. (Tayler & Hancock, 1975 ; Kalb, Chaouat & Chassoux, 1984).

Some workers have suggested that the HLA-alloantibodies are mostly absent in case of the women with recurrent abortions (Beer et al., 1981 ; Stimson & Blackstock, 1975 ; Rocklin et al., 1976 ; Ahrons 1971 ; Tiilikainen et al., 1974 ; Harris and Lordon 1976; Mowbray et al., 1983 ; Beard et al., 1984 ; Gelabert et al., 1981 ; Revillard et al., 1973). Thus, it is suggested that these antibodies are possibly necessary for the maintenance of the foetal allograft and their presence might predict a satisfactory outcome of the pregnancy. Beard et al., (1984) demonstrated that if the IgG fraction is removed from the serum, the protective effect will significantly reduce. Several workers have demonstrated that the inhibitory action of human pregnancy serum could be due to IgG,

present as immune complexes (Nakamura et al., 1983; Jajino et al., 1983; Voisin, 1983 ; Vanderbeeken et al., 1990; Stimson, 1980)

Beer et al., (1981) have demonstrated that the blocking activity is not directed against the antigens of HLA-A, -B or -C as the appropriate absorption studies with platelets carrying the -A, -B and -C locus antigens did not remove the blocking activity. This has indicated that the responsible antigen system is most likely associated with or determined by the HLA-D locus. Women with spontaneous abortions who shows a normal degree of migration inhibition in the presence of autologous antigen and in when no blocking factor is present in the sera may possess cytotoxic lymphocytes. These unsuppressed cells may focus attention on the foetoplacental unit, inciting either direct damage or indirect damage by releasing inflammatory mediators known to be incitors of premature labour.

In humans, very little is known about the pathogenesis of many couples exhibiting recurrent spontaneous abortions (RSA), placental abrouptions on foetal growth retardation. In studying HLA incompatible mating and spontaneous abortion, Lauritsen et al., (1976) found no significant differences from the frequency, but they have shown significantly depressed MLR in abortion prone mothers on stimulation with the father's lymphocytes, On the other hand several other workers have shown significantly higher frequ - ency of HLA compatibility in couples with recurrent abortion with

history of unknown etiology than in fertile couples (Komlos et al., 1977 ; Gerencer et al., 1978 & 1979 ; Beer et al., 1981 ; McIntyre & Faulk, 1983 ; Gerencer & Kastelan, 1983 ; Beer et al., 1985 ; Unander & Olding, 1983 ; Thomas et al., 1985 ; Reznikoff Etieavant et al., 1984 ; Aoki 1982 ; Sehacten et al., 1984 ; Caulan et al., 1987). On the contrary, several authors were not able to find out any significant HLA compatibility in RSA couples (Mowbray et al.,1987 Purpura et al., 1980 ; Caudle et al., Oksenberg et al., 1983 ; Vanoli et al., 1985 ; Cauchi et al., 1988).

Many investigators have suggested that association between HLA and recurrent abortion is not a direct influence of this region, but is an experession of the effect of a locus in the same chromosome, analogous to the T/t locus of the mouse (Rapaport et al., 1979 ; Fabio et al., Svejgaard et al., 1975 ; Bennet, 1975 ; Beer et al., 1981). The existence of such a locus in human is strongly suggested by the non-random associations or linkage disequilibria between the complotypes and the other loci of HLA complex in Caucasian families (Awed et al., 1983). The system is effective when the association is with a disease that is homologous from the pathogenetic point of view and when it is possible to hypothesize a direct and genic effect, as for instance in ankylopoietic spondylitis (Geezy et al., 1983 ; Dawkins et al., 1981).

Beer et al., (1991) demonstrated that the successful immunoregulation during pregnancy involves the presence of large

granulated lymphocytes in the bone marrow-like decidua. These lymphocytes have natural killer, natural suppressor and growth promoting cytokine activities. The signal for their migration to the uterus is unknown but may involve a foetal, truncated class-I molecule (HLA-G) in humans (Kovates et al., 1990). Beaman and Hovensland (1988) and Ribbing et al., (1988) have identified a T-cell suppressor cytokine produced in large quantities by lymphocytes harvested from the uterine draining lymph nodes very early in pregnancy during the preimplantation period.

The relationship between ABO incompatibility and abortion has been investigated (Szulman, 1973 ; Szulman, 1980) mainly by epidemiological studies, and there is a large body of evidence to support the existence of such a relationship. This correlation is interesting in that the trophoblast does not express ABO antigens.

There is conflicting evidence for a specific relationship between group O mothers and an increased prevalence of abortion (Takano et al., 1972 ; Brackenridge et al., 1979), but the study by Takano and Miller (1972) supporting such a relationship was the more convicing one. The cause of the abortion in the ABO-incompatible pregnancies may be the passage of antibodies directed against foetal blood group substances elicited in the mother by the transplacental passage of foetal cells and their interactions with the developing conceptus leading to the

disruption of organogenesis (Szulman, 1980). This is more likely to happen in group O mothers, because they produce IgG antibodies to blood group substances A and B rather than IgM antibodies, which are generally formed in group A or group B mothers (Szulman 1973 ; Szulman, 1980 ; Rawson et al., 1960 ; Mollison, 1979). There also appears to be a relationship between ABO incompatibility and trophoblastic neoplasia (Bagshawe et al., 1971), since group A women married to group O man are at the highest risk, where as group O women married to group A men are at the lowest risk.

Bauer et al., (1980) suggested that in couples having habitual abortions i.e., recurrent fetal loss in the first trimester in a women with the same, the wives share MHC antigens with either husbands at two loci in 20% of the cases, whereas normally fertile women share two MHC loci with their husbands only 8% of the time. Conversely, women having abortions share one on fewer HLA antigens with their husbands 76% of the time, but normally fertile couples share one or fewer antigens 92% of the time.

Changes in the balance between $CD4^+$ (helper/inducer) and $CD8^+$ (Suppressar/cytotoxic) T-cells have been demonstrated in allograft rejection and other disorders of presumed immune aetiology in man (Bach et al., 1980 ; Moromito et al., 1980 ; Cosimi et al., 1981). The percentage of T-cells in normal pregnancy has been variously reported as decreased or unchanged Siegal & Gleicher, 1981) and T-cells bearing Fc-receptors for immunoglobulin (IgG) may be increased (Sumiyoshi et al., 1981).

The relative proportions of various lymphocyte subsets defined by using monoclonal antibodies have more recently been reported in normal pregnancy (Vanderbeenken et al., 1982). This study showed a significant reduction in the T-cells due mainly to a fall in $CD4^+$ (helper/inducer) cells in normal pregnant women. $CD4^+$ (helper/inducer) cells were increased in unsuccessful pregnant women resulting in a slightly higher total T-cell count.

Kim et al., (1980) demonstrated that sera from multiparous women contained antibodies reactive only with activated PHA-stimulated T-cells. This antibody had no reactivity with resting T or B-cells. The authors concluded that this antibody, seen consistently in the sera of all pregnant or recently pregnant women, defines developmental antigens presented to the immune system of the mother during pregnancy. Their studies have been confirmed by Konoeda et al., (1986) who analysed public epitopes from serum isolated from 50,000 placentas, These epitopes were as immunogenetic as the private HLA class I epitopes, as determined by the frequency with which antibodies were produced against them. It will be very interesting to determine whether matching of public epitopes with antisera now available between organ donor and host will improve allograft survival in surgical transplantation.

Recent studies have provided some foundations for the belief that the mother's tolerance of the foetus is due to multifactoral relationship, including changes in the mothers immune

apparatus , changes in the subpopulations of the circulating lymphocytes and in their functions and regulating serum factors.

Few works have been done with pregnant women's T-cells treated with phytohaemaglutinin (Blecher et al., 1976 ; Fin et al., 1972 ; Hirano et al., 1977 ; Purtilo et al, 1972 ; Ringden et al., 1978), and this system has been considered as model to analyse the basic mechanisms involved in the activation of lymphocytes after binding with mitogen. Ortiz (1978) and his co-workers have confirmed the presence of a factor(s) in pregnant women's serum which is able to inhibit PHA induced human lymphocytes transformation in in vitro. Blecher et al., (1976) have shown that the lymphocytes response in the presence of PHA is depressed towards the pregnancy and increased again after delivery. The reduction in maternal lymphocyte responses to PHA in pregnant women could result from the specific action of blocking antibodies. Depressed maternal responses in mixed leucocyte cultures from pregnant women has been reported (Purtilo et al., 1972), and the responses of lymphocytes from non-pregnant women to PHA were reduced by incubation with 20% serum from pregnant women.

Most of the early studies on lymphocyte activation were carried out using either very impure preparation of PHA (PHA-M) or a partially purified fraction (PHA-P) prepared by the method of Rigas and Osgood (1955) from the Plant Phaseolus vulgaris (red kidney bean). PHA is a tetramer and composed of subunits with

molecular weights variously estimated at between 29,000 and 36,000 (Allan and Crumpton, 1971 ; Oh and Conard, 1972). Two different types of subunits were found, one of which had all the mitogenic activity, while the other was responsible for erythro-agglutination. Crude PHA contain some carbohydrate residues, mainly mannose and glucosamine (Weber, 1969 ; Allan et al., 1969). They also contain Ca^{++} and rather smaller amounts of Mn^{++} (Galbraith and Goldstein, 1970). The sequence of aminoacid residues at the N-terminal ends of both the erythroagglutinating and nitrogenic subunits has been determined by Edman degradation method (Miller et al., 1973). The two sub-units are different at six of the seven amino acids at the N-terminal end, but their sequences from residues 8 to residue 24, the last amino acid determined, are identical.

Specific sensitization to foetal HLA, resulting in altered maternal cell-mediated immune (CMI) responses, has been reported in human pregnancy (Rockin, Kitzmiller & Garovy, 1982). If much sensitization does occur, maternal lymphocytes would be expected to give a secondary MLR response pattern to patternal cells which bear the foreign HLA haplotype (Bonderik & Thorsby, 1974).

Some workers (Stimson, 1976 ; Damber et al., 1975 ; Johannsen et al., 1976 ; Ceri, Tatra & Bohn, 1977 ; Contractor & Davies , 1973 ; Murgita et al., 1978 ; Yachnin & Lester, 1976 ; Morse et al., 1976 ; Caldwell, Stites & Fudenberg, 1975) have suggested that some compounds (Pregnancy associated α_2 - glyco-

protein, pregnancy-specific β_1- glycoprotein, human placental lactogen, alfa-foetoprotein and human chrionic gonadotrophin) may contribute to the regulation of the maternal immune response to the foetus. But the actual biological functions of the majority of these remain obscure.

Several hypothesis have been proposed regarding the reduced immunological reactivity of the pregnant mother's sera and non-specific immune suppressive factors have been shown during pregnancy by studies with : mixed leucocyte culture (Kazakura, 1971 ; Gatti ; Yunis & Good, 1973 ; Jones & Gursen, 1973 ; Revillard et al., 1973) Blast formation induced by PHA(Purtillo, Hallgren & Yunis, 1972 ; Finn, Hill & Govan, 1972); rosette test (Stimson & Blackstock, 1975) ; macrophages migration inhibition (Rocklin et al., 1976),

Most immunological studies of abortion have looked during pregnancy for differences between women who abort and women whose pregnancies are maintained. This approach has led to the identification of various 'blocking factors' which are reduced in or absent from women who abort (Unander and Lindholm, 1986 ; Fizet et al., 1983 ; Power et al., 1983). Certain blocking activities have been reported to be associated with the maintenance of normal pregnancy (Faulk et al., 1974 ; Rocklin et al., 1976) which are absent from the blood of chronic aborters (Rocklin et al, 1976 ; Stimson et al., 1979). McIntyre and Faulk, (1982) have suggested that allotypic trophoblast - lymphocytes cross- reactive (TLX) antigens may serve

to stimulate mothers to mount blocking responses to their blastocytes, Sharing of TLX antigens between matting partners would thus seriously impair such blocking activity (McIntyre & Faulk, 1982) and result in poor prognoses for foetal survival (Faulk et al., 1982)

Relatively few immunological investigations (HLA alloantibodies serum Igs and cell mediated immunity) have compared normal pregnant women and unsuccessful pregnant women and conflicting findings have been reported.

So, it is apparent that a detailed study investigating the frequence of HLA antigens (both class-I and class-II), incidence of HLA alloantibodies, presence of serum immunoglobulins and in vitro cell mediated immune response of both normal and unsuccessful pregnancies would help in understanding the role of immunogenetic factors in unsuccessful pregnancies. Most of the studies carried out so for were in retrospect on smaller sample size making statistical considerations difficulty. Further, in most of these studies, the HLA-DR locus has not been intensively studied and no attempt has been made to corelate the HLA-A, -B, -C, -DR alloantibodies, serum Igs (IgG and IgM) and the cellular immune responsiveness withthe pregnancy outcome.

So, the present study has been designed to investigate the following aspects : i) to evaluate the incidence of HLA specific lymphocytotoxic antibodies (HLA-A, -B, -C and-DR)

in normal and in unsuccessful pregnancies i.e., those with spontaneous or repeated abortions, pregnancy induced hypertension (Pre-eclampsia) and women with premature delivery. ii) to understand the role of immunoglobulins and HLA alloantibodies in pregnancy out come and iii) to evaluate the cell mediated immune response including the ratio of $CD4^+$ and $CD8^+$ T-cells in both non pregnat (virgin), normal pregnant and unsuccessful pregnant women.