

# EVALUATION OF IMMUNE RESPONSIVENESS IN CASES OF ABORTIONS

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## Introduction

Transplantation immunology by recent advances has considerably stimulated the interest in immunological aspect of pregnancy, where foetus exists as a well-tolerated homograft. Since the transplanted zygote is a sort of an alien antigen it is but natural for the immunological apparatus of pregnant mother to react to it. The depression of cell mediated immunity and decrease in the number of T lymphocytes has been reported by Finn *et al* (1972).

Keeping in mind an altered immunological basis of pregnancy, the present study is undertaken to postulate the abnormality of immune responsiveness in females of various types of abortions.

## Material and Methods

The study included investigations for the assessment of immunological status of 13 normal first trimester pregnant females as control and 46 females with various types of abortions.

Following is an account of tests employed for the assessment of the immunological status of the cases under study:

### A. Lymphocyte Studies.

1. Peripheral blood absolute lymphocyte count (P.L.C.) as described by Dacie and Lewis (1969).

2. T-lymphocyte (E. rosettes) percentage and levels as described by Jondel *et al* (1972).

3. B-lymphocyte (E.A.C. rosettes) percentage and levels. As described by Pincus *et al* (1972).

### B. Skin Test Studies

Delayed cutaneous hypersensitivity reactions were performed by using recall antigens Dermatophytin 'O' (an extract of candida antigen) and P.P.D.

### A. Lymphocyte Studies

1. Peripheral blood absolute lymphocyte count (P.L.C.)

It was obtained by multiplying the percentage of lymphocyte and total leucocyte count taken in hundred.

### 2. Lymphocyte Subpopulation Studies Separation of Lymphocytes

About 4 ml of blood was collected in vial containing 100 I.U. heparin. Lym-

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phocytes were separated by layering 3 ml of undiluted blood on 1.5 ml of Ficoll Hypaque mixture (obtained from Nygaard & Co. Norway lot No. 70479 as lymphoprep) and centrifuged at 400 g. for 10 mts at 4°C. The condensed layer of lymphocyte at the interface of lymphocyte and supernatant plasma was taken in another test tube and cells were washed twice in Hanks balanced salt solution (PH 7.2-7.5). The third washing was done with minimum essential medium (PH 7.2-7.5) containing A.B. serum in a ratio of 9:1. Viability was tested with 1% trypan blue and count of viable cells was adjusted to  $4 \times 10^6$  cells/ml.

*Assay for T Lymphocytes (E. Rosettes)* (Jondal *et al* 1972 with slight modifications).

0.25 ml of peripheral blood lymphocyte was mixed with equal volume of .5% washed R.B.C. suspension and centrifuged at 200 g. for 5 mts at 40°C and kept at 4°C for 3 hours. The cells were suspended gently and fixed in .6% glutaraldehyde solution. The cells were stained with .5% methyl violet. Two hundred lymphocytes were counted and percentage of E rosettes (lymphocyte with 3 or more erythrocytes attached) was recorded. Total T lymphocyte level was calculated with help of T lymphocyte percentage and absolute lymphocyte count.

*Assay for B Lymphocyte (E.A.C. Rosettes)* (Pincus *et al* 1972 with slight modifications).

#### *Preparation of E.A.C.*

Amboceptor (Anti sheep haemolysin 1: 4000 lot No. 2016 c) and lympholysed guinea pig compliment (lot No. 3221 C) along with its solvent (lot No. 3320 K) were obtained from M/s. Behrinwereke A.C.W. Germany).

Sheep R.B.C. having a concentration of

$5 \times 10^8$  cells/ml were treated with equal volume of diluted amboceptor (1:750 diluted with P.B.S.) incubated at 37°C for 30 mts. Following incubation the cells (EA) were washed with Hanks balanced salt solution twice and treated with diluted guinea pig compliment (1:1000 diluted with P.B.S.) and incubated at 37°C for 30 mts, the cells (E.A.C.) were washed twice with Hanks balanced salt solution and final solution having sheep R.B.C.  $5 \times 10^8$  cells/ml was obtained in M.E.M.

#### *E.A.C. Rosettes*

Equal volume of E.A.C. (0.5 ml) suspension was mixed with equal volume of lymphocyte suspension ( $4 \times 10^6$  cells/ml) and incubated at 37°C for 5 mts, then centrifuged at 300 g. for 5 mts. The cells were thoroughly mixed and examined in the same way as E. rosettes. The B cell percentage was obtained and later B cell level was calculated.

#### *Skin Test Studies*

*Dermatophytin 'O' skin test:* Under precautions 1:100 diluted antigen was prepared in P.B.S. (p.h. 7.2). After sterility testing, .02 ml was injected I/L on the flexor aspect of left arm. Reading was made after 48 hours and area of induration measuring more than 5 mm in diameter was taken as positive (Wanebo, 1975).

*P.P.D. skin test:* (Fudenberg *et al* 1976) 0.1 ml of 1:1000 diluted old tuberculin was injected intra dermally and reaction was measured after 48 hours. Area of induration measuring 10 mm or more was taken positive.

#### *Observations*

##### *A-Lymphocytes Studies*

The range, average and variation of P.L.C./cu mm. T. cell%, total T cell level,

TABLE I  
*Study of T Lymphocytes in various Abortions and 1st Trimester Normal Pregnancy*

Type of Cases	No. of cases	PLC/cu.mm Range Mean S.D.	T Cell % Range Mean S.D. %	T Cell level/ cu.mm. Range Mean S.D.
1. 1st trimester normal pregnancy as control	13	(1520-3120) Mean—2456.08 S.D. $\pm$ 431.66	(22-28) Mean—27.38 S.D. $\pm$ 4.31	(456-904) Mean—665.08 S.D. $\pm$ 121.57
2. Spontaneous abortions P. Value	18	(2133-3680) Mean—2783.89 S.D. $\pm$ 442.47 P > .05	(50-62) Mean—58.44 S.D. $\pm$ 4.22	(1066-2230) Mean—1701.11 S.D. $\pm$ 1377.74 P < .001
3. Threatened abortions P. Value	18	(1792-3528) Mean—2699.33 S.D. $\pm$ 630.37 P > .05	(22-30) Mean—27.55 S.D. $\pm$ 3.00	(473-1020) Mean—747 S.D. $\pm$ 207.73 P > .05
4. Missed abortions P Value	10	(2150-3682) Mean—2981 S.D. $\pm$ 601.75 P > .05	(55-70) Mean—63.8 S.D. $\pm$ 6.94	(1505-2224) Mean—1926.20 S.D. $\pm$ 1377.07 P < .001

TABLE II  
*Study of B Lymphocytes in various Abortions and First Trimester Normal Pregnancy*

Types of cases	No. of cases	B-cell% Range Mean S.D.	B-cell level/cu.mm Range Mean S.D.
1. First Trimester Pregnancy as control	13	(31-39) Mean—35 S.D. $\pm$ 3.04	(732-1073) Mean—916.69 S.D. $\pm$ 124.83
2. Threatened abortion P. Value	18	(31-39) Mean—33.33 S.D. $\pm$ 2.43	(732-1073) Mean—893.39 S.D. $\pm$ 121.65 P > .05
3. Spontaneous abortions P. Value	18	(10-15) Mean—12.89 S.D. $\pm$ 1.40	(254-401) Mean—320.11 S.D. $\pm$ 47.89 P < .001
4. Missed abortions	10	(8-13) Mean—10.5 S.D. $\pm$ 1.5	(252-352) Mean—285.4 S.D. $\pm$ 27.41 P < .001

B. cell% and total B level in control first trimester normal pregnant females and in cases of abortions is shown in Tables I and II.

There is no change in P.L.C. in various abortions as compared to first trimester normal pregnancy.

It is evident that in cases of threatened abortions, no change in P.L.C. T cell percentage and levels, B cell percentage and levels was observed as compared to controls ( $P > .05$ ).

In cases of spontaneous abortions and missed abortions T cell percentage and levels were increased significantly ( $P < .001$ ) when compared with normal controls. On the other hand, B cell percentage levels were significantly decreased ( $P < .001$ ).

#### B-Skin Test Studies

The cutaneous reactivity with dermatophytin 'O' and P.P.D. is illustrated in Table III. It is evident that in cases of spontaneous and missed abortions increased percentage of positivity to these antigens was observed as compared to normal controls, whereas near to normal control values were obtained in cases of threatened abortions.

In cases of spontaneous and missed abortions, there was a significant rise ( $p < .001$ ) in T cell percentage and levels when compared with normal control, thereby indicating an enhancement of cell mediated immunity in these group of cases which might be responsible for an early rejection of foetal allograft. Similar findings were observed by Helbrecht and Kolmos (1968) who found and increased number of transformed lymphocytes in the mixed lymphocytic culture of wife and husband. The increased cell mediated immunity in cases of abortions is also supported by increased rate of rejection of skin transplants from the heterologous donors (Bardawil *et al*, 1962).

On the other hand, in cases of threatened abortions no significant change was found in any of the parameters. This indicated no change in the cellular immune response that might be responsible for the continuation of the pregnancy.

While studying the B cell levels in comparison to controls, no significant change was found in cases of threatened abortions ( $P > .05$ ). On the other hand, in cases of spontaneous and missed abortions the B cell levels were significantly lowered ( $P < .001$ ) as compared to normal

TABLE III  
Skin Test Study in First Trimester Normal Pregnant Females and Cases of Abortions

Types of cases	No. of patients	P.P.D.		Dermatophytin 'O'	
		+ve	-ve	+ve	-ve
1st Trimester	13	38.46%	61.54%	15.38%	84.3%
Threatened abortions	18	44.44%	55.56%	11.11%	88.89%
Spontaneous	18	44.44%	55.56%	22.22%	77.78%
Missed	10	60%	40%	80%	20%

#### Discussion

Evaluation of immune response in various abortions revealed variable results.

This indicated fall of humoral immunity which may be responsible for the absence of blocking antibody related to IgG chemically. Rocklin *et al* (1976)

and Stimson and Blockstock (1976) demonstrated the absence of blocking factor in cases of abortions. Hellstrom (1970) and Than *et al* (1974) suggested that in normal pregnancy rise in B cell level may assist in allograft acceptance through production of blocking antibodies or factors, while may be responsible for decreased production of blocking factors thus causing rejection of foetal allograft.

#### Summary

The immune response was evaluated in 46 females with abortions, which included 18 cases of threatened abortions, 18 cases of spontaneous abortions and 10 cases of missed abortions. The findings were compared with 13 first trimester normal pregnant females who served as control cases. T (E. rosettes) and B (E.A.C. resettes) lymphocyte levels were determined and the findings were correlated with delayed cutaneous hypersensitivity reaction to recall antigen. Dermatophytin 'O' and P.P.D. In cases of threatened abortions, no significant change in T cell levels and B cell levels were observed as compared to normal controls. In cases of spontaneous and missed abortions, T cell levels were significantly increased and B cell levels were significantly lowered as compared to normal controls. Skin test also revealed increased positivity as compar-

ed to controls in all cases of abortions. Thus it was concluded that in cases of spontaneous and missed abortions there is enhancement of cell mediated immunity which may be responsible for early rejection of foetal allograft.

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