

UNEXPLAINED INFERTILITY AND ABO BLOOD GROUP INCOMPATIBILITY

by

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A difficult diagnostic problem arises when apparently thorough investigations by gynaecological and urological standards fail to reveal any definite cause of infertility in the couple. The husband and the wife appear to be normal but nevertheless conception does not take place; this unknown or refractory infertility constitutes a variable percentage among the infertile couples.

It has been fairly well established that incompatibility in the ABO matings is responsible for foetal wastage through miscarriage, stillbirth and haemolytic disease (Levine, 1943). The implication of blood groups in the selection of the neonates has been studied in recent years by many workers, Sanghvi (1951), Cohen and Glass (1956), Johnstone (1954), Sheild *et al.*, (1958), Banerjee *et al.* (1963) and others.

That serological incompatibility might be a cause of infertility is comparatively a recent trend of thought. Though in the beginning of this century, Landsteiner (1899), Metchinkoff (1900), independently discovered that sperm immobilising anti-

bodies could be induced in female guinea-pigs by intraperitoneal injection of heterozygous (bovine) sperm (Schwimmer *et al.*, 1967). But only during the last 20 years, the immunological factors that affect human infertility has been elaborated by several workers (Rumkee, 1954, Wilson, 1956; Matsunaga and Itoh, 1958; Rao and Sadri, 1959; Behrman and colleagues, 1960; Franklin and Dukes, 1964; Boettcher and Hay 1968; Isojima and Tsuzuku, 1968 and Fjallbrant, 1968).

In spite of all the works mentioned above our knowledge with regards to serological incompatibility and infertility is not yet very clear and consequently it is yet to be known thoroughly whether the difference in blood groups between the couples can be accounted as a contributory factor in the causation of disturbed fertility (Nag and Banerjee, 1970).

Keeping in mind about the problem of sperm inactivation an attempt has been made to find out the role of ABO blood groups incompatibility in primary infertility.

Material and Methods

Since 1966, the effects of ABO and Rh blood group incompatibilities on reproductive performances were determined in the Genetic laboratory of this hospital. The study included normal fertile couples, primary and secondary infertile couples

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and women who had miscarriages, stillbirths and where the children were affected by haemolytic jaundice. As the work is still being continued, the present communication deals with the result of three years' study (1967—1969).

Total number of couples included in this investigation was 90 who had no demonstrable organic cause of primary infertility. The minimal criteria for the selection of the couples for the present study were couples living together for at least 2 years without any contraceptive practice, with normal pelvic findings, normal insufflation test (often hysterosalpingography done), endometrial biopsy in late second half of menstrual cycle (ovulatory phase) and normal semen analysis of the husband. An essentially normal pertinent blood study (haemoglobin estimation etc., V.D.R.L., blood sugar) of the partners.

Nearly 2 ml. of blood was drawn from each partner by vein puncture. The samples were tested for ABO and Rh(D) types according to the standard methods (Boorman and Dodd, 1970). The status of the mating types were ascertained after Levine (1958). Arrangements were made to collect semen inside the hospital. Complete semen analysis were performed by a standard method which included volume, Ph total sperm count, number of motile and abnormal forms and pus cells. Substandard semen quality was not accepted for this study and the particular investigation was deferred till the improvement of the semen quality.

Finally, the semen of the husband was tested against the serum of the wife on a slide under the microscope for the evidence of sperm immobility and agglutination. The test for agglutination was also performed in serological tubes by adding 0.05 ml. of the suspension of

sperm to 0.5 ml. of the wife's serum, undiluted and diluted in 1:5 isotonic saline. The tubes were then incubated for 4 hours at 37°C. The readings were then noted at an interval of 30 minutes, first, second, third and fourth hours against the control sample. Evidence of positive agglutination was considered to be aggregation of two or more spermatozoa per high power field within 4 hour test period. Evidence of strong agglutination was the aggregation upto 10-12 sperm cells per aggregate in numerous aggregates per high power field. In general head to head agglutination was the most common finding and a mixed agglutination of head to tail was rare.

It was noted that some highly motile sperm when came in contact with compatible serum (wife's or from stock) remained motile but the motility immediately was affected when it came in contact with serum of the incompatible blood group and even agglutinated instantaneously with incompatible serum.

Results

The incidence of ABO blood groups among the husband and the wife of the infertile couples have been presented in Table I and the compatible and incompatible mating types have been presented in Table II. The findings of the serum-semen reaction among the ABO incompatible couples have been shown in Table III.

It is evident from the above Tables that in majority of the cases (66.67%), restricted mobility of the sperm in the presence of incompatible serum were noticed, while in 8 (14.03%) cases there were fair to strong agglutination reaction. In 11 (19.3%) cases however there were no sperm immobilising reaction. Among the above mentioned patients, the frequency

TABLE I
Distribution of ABO Blood Groups Among the Infertile Couples

Husband's blood group	Wife's blood group				Total
	O	A	B	AB	
O	8	4	9	1	22
A	12	1	10	1	24
B	16	10	7	1	34
AB	4	1	4	1	10
Total:	40	16	30	4	90

TABLE II
ABO Mating Types Among the Infertile Couples

ABO Compatibility			ABO Incompatibility		
Wife	Husband =	Total	Wife	Husband =	Total
O	O =	8	O	A =	12
A	O =	4	O	B =	16
A	A =	1	O	AB =	4
B	O =	9	A	B =	10
B	B =	7	A	AB =	1
AB	O =	1	B	A =	10
AB	A =	1	B	AB =	4
AB	B =	1			
AB	AB =	1	Total:		57
Total:		33			

TABLE III
Result of Serum-Semen Reaction (sperm agglutination) Among the ABO Incompatible Couples

ABO Incompatible	No reaction	Restricted mobility	Positive agglutination	Total
Wife Husband				
O A	3	7	2	12
O B	3	13	-	16
O AB	-	3	1	4
A B	3	6	1	10
B A	-	1	-	1
A AB	1	7	2	10
B AB	1	1	2	4
Total:	11 (19.30%)	38 (66.67%)	8 (14.03%)	57 (100%)

of auto-agglutination appeared in 4 per cent cases. Low titre might be the cause of the non-reaction, while the heterozygotic nature of the husband was not ruled out.

compatible groups, none showed any trace of restricted movement of the sperm or agglutination reaction.

It was interesting to note that in the follow-up study, 14 (15.5%) women in the series of 90 primary infertile women

In contrast to the above findings, in the

conceived. Nine out of 38 in the compatible group and 5 out of 57 women in the incompatible group had conception. In the latter group, 3 women from no-reaction and 2 from restricted sperm movement group were pregnant, but none conceived from positive agglutination group.

Discussion

Blood group antigen has been found on the sperm (Edwards *et al*, 1964; Boettcher, 1968), while the blood group antibodies have been detected in cervical mucus (Behrman *et al*, 1960). It is quite possible that a fraction of the sperm is inactivated by the antibody of the cervical mucus and thereby conception is prevented. This of course will be dependent upon the relative quantities of the sperm and the antibodies present at the time of insemination and since there are ordinarily large number of sperms available and hence in most cases there would be no antifertility effect but low fertility cannot be ruled out.

It is believed that the wife has a natural occurring ABO antibody which is not specific for husband's serum. Whether the husband and wife are homozygous or heterozygous must also be considered. The presence of secretor trait of the various body fluids has also been believed by some investigators. Franklin and Dukes (1964, a and b) from a reasonable series of infertile couples found that sperms were agglutinated by the wife's serum in incompatible matings. The antibody has been found to be heat-stable, to reside in the gamma-globulin fraction of the serum. The circulating sperm-agglutinating antibody may only be a reflection or indicator of the immunological process involved not specifically responsible for the state of infertility.

There are some difference of opinions present amongst the workers on this subject as Whitelaw and co-workers (1962), did not find any relation of ABO incompatibility with infertility. Solish (1969), thinks that if cervical agglutinins play any role in the process of fertilisation it is a more complex one than previously supposed. Boettcher and Hay (1968), remarked that 'it seems that an interaction between ABO antigen on sperm and ABO antibodies in cervical mucus occurs in some women, but it is concluded that there is no sound evidence that such an interaction leads to infertility'. But, Segal and co-workers (1961), found sperm agglutinating antibodies more frequent in subfertile couples. Tyler and colleagues (1967), found statistically significant antifertility effect due to ABO incompatibility.

During the period of investigation, only 14 females conceived, mostly with compatible mating and 5 with incompatible mating status. It is an interesting point to note that none of the patients who had shown the serum-semen agglutination conceived. The result indicate that however the small number of cases may be, the antispermatozoal antibody most likely have effected fertilisation among the couples who have shown positive result in the serum-semen agglutination.

There is a suggestion that ABO antigen lies on the surface of the sperm as a superficial coating which is weak in nature. Dukes and Franklin (1968), found that when couples were persuaded to abstain sex relationship or restrict the intercourse and specially use condoms during the act from 2 to 6 months, the sperm agglutinating antibody titres decline in women, often drop to non-detectable level. Fertilisation can be achieved in these couples at the time of ovulation.

In some of our cases conception occurred following the above technique but all of them were from restricted mobility group and none of them from positive agglutination group, although couples from both groups adopted the same procedure.

Summary and Conclusion

Antispermatozoal antibody in women causing primary infertility is a new concept. During three years, 90 couples where no apparent cause of infertility could be detected by usual investigations were subjected to the ABO blood group study, which revealed that 57 couples had incompatibility and 33 had compatible mating status.

In the incompatible group, sperm-serum (wife's) reaction was studied up to 4 hours. In 8 (14 per cent) cases positive agglutination was noted. In 38 (66.67 per cent) cases restricted sperm movement was noted and in 11 (19.3 per cent) of the cases no change of the sperm activities were detected.

A follow up study upto one year, revealed that 14 women conceived, of which 5 were from the incompatible group. Of these 5 women, 3 from no-reaction group and 2 from restricted sperm movement group. Conception did not occur in agglutination positive couples.

The results suggest that spermatozoa can possibly provide an antigenic stimulus resulting in elaboration of the circulating antispermatozoal antibody in some women. Though the number is small in the present series yet there is ample evidence to correlate the existence of antispermatozoal antibody in women to account for the unexplained infertility.

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