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Obstetrical immunologic incompatibility

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A) INTRODUCTION

The over-all incidence of infertility in the United States is thought by various authors to be between eight and twelve percent, but may range as high as twenty-five percent. This incidence varies with age and markedly rises after the age of thirty. Infertility is defined as, "eighteen months of adequate exposure without the use of contraception." Primary infertility is the term used to designate those patients who have never conceived. Secondary infertility indicates that the patient has had one or more pregnancies and fails to conceive again.

Guttmacher[12] shows evidence that the incidence of infertility varies with age. Marriages of women sixteen to twenty have a 4.5 percent infertility rate, while the incidence is 31.3 percent for married women between the ages of thirty-five and forty. After the age forty the infertility rate is thought to be seventy percent.

The concern of this paper will be with one of the causes of primary infertility which was postulated by Behrman[3] in 1961, that of immunologic incompatibility.

B) ETIOLOGY OF INFERTILITY

Stevenson[36] states that the etiology of infertility may be separated into four groups and by percentage basis.

1. Hypo-Ovarian Group.....51%
2. Hypothyroid Group.....32%

3. Blocked Tubes Group.....10%

4. Miscellaneous..... 7%

Behrman[5] states that thirty to forty percent of infertility is due to the male partner; forty-five percent to the female partner, and ten to fifteen percent to the couple as a unit. His division of infertility as to the female partner, with which we are concerned includes:

1. General
    a) Obesity
    b) Severe Anemia and Debilitating Disease
    c) Psychologic (Anxiety and Fear)
2. Development and Endocrine
    a) Uterine Hypoplasia
    b) Uterine Anomalies
    c) Gonadal Dysgenesis
    d) Polycystic Ovarian Disease
    e) Pituitary Failure
    f) Hypo or Hyperthyroidism
    g) Adrenal Hyperplasia
    h) Ovarian Failure
3. Genital Disease Process
    a) Pelvic Inflammation
    b) Endometriosis
    c) Fibroids and Polyps
    d) Vaginitis

e) Cervicitis

f) Carcinoma

4. Unexplained

a) Lack of receptivity of cervical mucus or immunologic incompatibility

C. STATISTICAL EVIDENCE

The fact that those who lack the blood group antigen A have anti-A antibody, and those who lack group B antigen have anti-B antibody leads in a number of cases to an incompatibility between maternal serum and fetal antigen inherited from the father. Previously such serologically incompatible pregnancies have been thought to be a possible cause of pathologic conditions in the fetus and mother.

Hirszfeld and Zborowski[13] in 1925 found that in the matings of father group A and mother group O there were relatively fewer group A offspring than in the reciprocal matings father O with mother A. It was also noted there were fewer children in the matings father B mother O than the reciporal father O mother B.

Waterhouse and Hogben[38] later analyzed a population of over 6,000 mothers and agreed with the preceding statements but estimated the loss of A children to A fathers and O mothers to be 25% and suggested that this loss was due to early abortions.

A later study by Kirk, Kirk, and Stenhous[16] suggested that this loss of 25% of group A children also accounted for a loss

of 3% of all conceptions.

It was from these studies and studies of others [1, 16, 17] that the homospecific or compatible matings, heterospecific or immunologically incompatible matings, and heterospecific pregnancy groupings were patterned. These groups are as follows:

Homospecific matings.

Male	Female
A	A
B	B
AB	AB
O	A, B, O, AB

Heterospecific matings.

Male	Female
A	O, B
B	O, A
AB	O

Heterospecific pregnancies.

Mother	Infant
O	A, B
A	B, AB
B	A, AB
AB	----

A subsequent study by Kirk, Kirk, and Stenhous[17] demonstrated that 100 group O, Rh positive women age 26-35 would have 36 (average) fewer children than 100 group A, Rh positive women of the same age. In comparable groups after age 40 however, the Group O women would have 16 (average) more children.

It was found that group O mothers experience more pregnancies

below age 20 and above age 35, but between these ages there is the previously mentioned decrease in the number of expected offspring.

Compensation for this could occur from:

1. Group O women having an inately higher fertility than group A women.
2. Group A females suffer a reduction in fertility rates later than group O females.
3. Group O women in heterospecific mating groups prolong efforts in attempts to have children and meet with more success after age 35.

In Japan there has been noted a 20% mortality rate among infants in heterospecific pregnancy groupings.

D. HISTORICAL REVIEW

Metchnikoff and Landsteiner independently and simultaneously in 1899 reported on the production of spermatotoxins. Metchnikoff injected rabbit testes extract into guinea pigs and discovered the serum of the guinea pigs acquired properties of rapidly immobilizing the sperm of rabbits without however, causing their dissolution.

Taylor in 1908 was the first to obtain an antisera following injection with whole sperm into the rabbit. The donor animal was a salmon.

Pittler in 1920 made intravenous injections of fresh ejaculate fluid into female rabbits and found that sterility could be

caused by such injections and that these injections didn't interfere with the estrous cycle of the female. He tried intravenous injection of horse sperm and found that the serum collected at intervals following this sensitization was specific for horses but didn't work in mammalian interchange.

McCartney found in his work in 1923 that female rats could be immunized for a period of 2-22 weeks by injection of the organ extracts of sperm and testes from male rats while extracts from other organs of rats didn't cause sterility. He also found that previously immunized females produced smaller litters.

Hektren and Manly in 1923 found that injection of human semen and human seminal fluid and extracts of these induced the formation of precipitins that were specific for human seminal proteins.

It was noted by Kohlbrugge in 1912 that there may be penetration through the germinal epithelium and invasion of underlying connective tissue of a female rat by spermatozoa following normal coitus.

The work of Waldstein and Ekler in 1913 led them to describe, "post copulatory ferments" which were absent before copulation that led to the absorption of sperm and a specific Abderholden reaction in female rabbits within 24 hours of copulation. (This is the reaction comparable to that given by the blood of a pregnant female to placental material.)

Mayer's work in 1922 concluded that sexual intercourse in females may lead to premature rupture of follicles and cause sterility due to the abortion of these follicles. He states that overloading the female genital tract with sperm could cause immunity. However, intercourse after a period of abstinence can result in pregnancy. Voght confirmed this conclusion one year later.

Pommerenke[28] in 1928 attempted further work on the penetrability of spermatozoa into the tissue of the female genital tract, where it was thought initially, they acted as an antigen inducing the formation of antibodies against sperm. At this point he noted that infertility for a period of weeks could follow repeated injections of sperm or testicular extract, but that rabbit salivary gland extract or fluid from the ejaculation of a vasectomized rabbit didn't cause sterility. His conclusion was that repeated intravaginal injection of rabbit sperm by natural or artifical means may produce antigenic effects in the blood or vaginal secretions.

It was in 1932 that Baskin[2] described the temporary sterilization of females by injection of human sperm. He concluded that immunization was tissue specific and that large quantities of antigen were necessary for immunization. His study involved injection of no less than 9 cc. of sperm into the buttocks, 3 3 cc doses at 7 day intervals. He demonstrated serum (immune)

properties from the blood of the females which immobilized the sperm.

The three different types of sperm agglutination that of head to head, tail to tail, and mixed were initially described by Henle in 1938.

An Italian author Bocci[6] in 1956 attempted to utilize a skin test performed with the sperm of husbands in their wives to test for infertility. He found the intradermal test was positive only 25% of the time and he concluded that no direct proof of the existence of a state of immunization could be shown.

Kiddy[15] in 1959 studied the question of whether female fertility can be reduced by immunization with sperm. His work was performed in rabbits. It was found that in rabbits with normal serum and normal sperm gave a fertility rate of about 91%, and that rabbits with high concentrations of immune serum fertilization was prevented. This immune serum was produced by innoculation with sperm into the muscle of female rabbits. Embryo survival decreased in litters from female rabbits with immune serum in low titers. The mechanism of fertility failure by antibodies was unknown. The effect on fertility couldn't be explained on the basis of gross amounts of sperm agglutination or any marked interference with sperm mobility.

E. THEORY OF IMMUNOLOGIC INCOMPATIBILITY

It was noted in the work of Gershowitz, Behrman and Neel[9] in 1958 that natural selection could possibly influence ABO

blood group phenotypes. This evidence was taken from their previous work,[3] Briefly, evidence demonstrated that:

1. Offspring from incompatible matings of mother type O father type A show a deficiency of offspring of type A groups.
2. The frequency of abortions is higher and the mean number of living children is lower in incompatible matings.

Mechanisms thought possible by these authors include:

1. Immune antibodies produced by the mothers could cause death or damage to the fetus.
2. Selection exercised on spermatozoa directly at pre-conceptional levels.

It is known that human spermatozoa have definite specific antigenicity and show antigenic dimorphism[11,35] which is not influenced by either secretor status or blood group status of seminal fluid. A previous assumption was made that the presence of soluble blood group substances in seminal fluid had been a determining agent in spermatozoal selection.

Elimination of sperm in the female reproductive system may be by inactivation, cessation of motility, destruction of blood group antigen or other as yet unknown mechanisms.

The attempt was them made to prove the presence of hemagglutins in cervical mucus or presence of a fixed tissue antibody mechanism in the female reproductive tract.

The initial detection of hemagglutins in cervical fluid was performed by Schwarzmann in 1928 on a single sample. Further detection of anti-A and anti-B agglutinins in cervical secretions of normal women was performed by Gershowitz[9].

Work performed by Rao and Sadri[30] in 1959 demonstrated the presence of antibodies in the cervical mucus to sperm antigens which lent support to the hypothesis of immunologic incompatibilities operating at a preconceptional level.

Gershowitz[9] et. al. in 1958 performed work to explore the quantitative levels of ABO agglutins in cervical mucus. In contrast to finding only anti-A and anti-B antibodies together in blood group O woman; these antibodies could also be found singly, either anti-A or anti-B alone in the cervical secretions of these type O women.

Evidence presented by Rosenfield and Ohno[31] suggest that isohemagglutins produced by Group O individuals differ from those produced by group A and group B persons. Support of this was shown by Rawson and Abelson who found that type O individuals produced isoagglutin compounds primarily of gamma 2 globulins of low molecular weight, and possessing other differentiating characteristics. It can be suggested from this work that perhaps a selective mechanism is at work to secrete antibodies of a given characteristic in body fluids or cervical mucus since they are derived from the blood.

Straus[37] in 1959 demonstrated a local antibody forming mechanism by application of soluble typhoid material to the vaginal mucosa. Similar selective mechanisms may occur from the presence of normal vaginal tract bacterial flora.

It may be postulated that the production of cervical hemagglutins could occur by one or three or more mechanisms:

1. Passive transfer from serum
2. Selective active passage from serum
3. Local production from previous stimulus

The initial work of Gershowitz[9] et. al. demonstrated that with random selection and use of only one sample, 23.7% of subjects demonstrated secretion of isohemagglutins while on a subsequent collection of numerous samples from the same patients the percentage rose to 63.4%. Blood group type O women had a higher incidence of agglutins in cervical secretions than either type A or B women. No correlation could be noted between secretion titers and serum hemagglutin levels. Occurrence of antibodies in cervical mucus was unaffected by secretor type. There was no noted correlation of titers to the phase of the menstrual cycle.

Behrman's[4] work in 1961 demonstrated a preponderence of incompatible matings in infertility; heterospecific matings being present in 87.3% of infertile couples as compared to 38.6% in fertile couples. In seven couples showing delayed fertility there were 9 children produced, all of blood type O from group A fathers

and group O mothers.

The work of Whitelaw and Grams[39] in California recently attempted to disprove the statistics and theories of the Behrman work. In their study they found only 42% of incompatible blood types as compared with Behrman's 87.3% in his series of infertile couples. A series of 100 women who received artifical insemmination with doners of an incompatible blood type yielded only a 33% failure of conception. A control group receiving compatible blood group doner matings had a failure rate of 36%. It was further noted in 136 patients of gravidia 4 or more that there was a 34.5% proportion of incompatible (Heterospecific) matings as compared to Behrman's 36% with comparable patients of gravidia 2 or more.

F. PHYSIOLOGY

1. Secretor Status

The blood group specific substances i.e. the antigens ABO(H) are secreted in all of the body secretions including the amniotic fluid with the exception of the cerebral-spinal fluid. The percentage of people in the general population who are secretors is thought to be from 78-80%.

These same group specific substances are found on the erythrocytes. These substances are divisible into two groups, the alcohol soluble antigens and the water soluble antigens. The presence of the water soluble substances is thought to be con-

trolled by a genetic mechanism.

This was thought originally to be a simple Mendelian dominant trait. A person belonging to group A secretes A substance in the saliva, B secretes B and O secretes a water soluble antigenic substance called "H". The dominant gene Se may affect the secretor status as either homozygous Se/Se (25%) or heterozygous Se/se (50%). The non-secretor is homozygous se/se (25%). Non-secretors produce a similar muco-protein substance which lacks the ability to neutralize anti-A, anti-B, or H.

The Se gene controlling secretor status is inherited independently of genes controlling ABO blood group status, there is no linkage. In the Lewis Blood system however Le^{W}(a-) are secretors and Le^{W}(a+) are non-secretors.[20]

Recent work by McNeil[22,23] adds complexity to this simple Mendelian dominant transmission theory of secretor status. It would found that most human secretors produce "H" as well as A or B. Also noted were populations of humans of blood group A and B who secrete "H", but not A or B, or secrete "H" in low titers, and A or B in more dilute titers (1:256).

This work indicates a more complex basis to the secretor status which is at the present time not understood. It is currently thought that the non-secretors may represent the suppression of a gene during one generation.

The point that should be understood is that while an individual is secreting any one of these substances he may be also

producing the antibody. An A secretor will produce anti-B antibody or isoagglutins and B secretors would produce anti-A isoagglutins. AB secretors would produce either "H" isoagglutins or none depending upon the secretor status present at any given time.

2. PROPERTIES OF CERVICAL MUCUS

J. Marion Sims in 1868 wrote, "In the investigation of a case of sterility if we expect to procede understandingly we must determine whether the secretions of the cervical canal are favorable or not to the vitality of the spermatozoa." This statement only emphasizes the importance of the cervical mucus at that time and today.

The first barrier the advancing sperm must meet and surpass is the viscous mucus secretion. It is an epithelial secretion 92-94% water at pre and post-ovulatory times, and 98% water at midcycle. At midcycle the mucus contains few leukocytes, is highly alkaline and most abundant. The average daily secretion is 60 mgm. Its composition is of mucopolysaccharides, glactose, and hexosamine with fucose and sialic acid. The nitrogen content is 12.1% while the total protein content is only 1%.

This 1% is the most important, for it is in this protein that the gamma globulin group is present which holds the apparent existence of the blood group antibody itself.

Protein composition is found electrophoretically to be 21%

albumin, 36% globulin, while 43% is a non-migrating component. The globulin fraction is significantly decreased during midcycle when there is exhibited maximum spinbarkeit and arborization. This fraction is composed of two alpha-2, three beta-1, and two beta-2 groups as proven by the excellent work of Moghissi.[25]

It has been shown that the cervical mucus proteins originate from the blood serum. To prove this, radioactive Iodine I_{131} human serum albumin (R.I.S.A.) was injected intravenously into human subjects and there appeared subsequently later radioactivity of the cervical mucus. The production of cervical mucus is not just simple filtration but possibly due to a selective capillary permeability associated with the cervical glandular epithelium, as shown by the absence of three alpha-1 and two alpha-2 globulin components normally found in the human serum globulins. It is a well known fact that these globulin electrophoretically defined protein zones contain blood antibodies including those of the ABO(H) system blood groups.

Straus[37] states that this antibody is not a protein antibody per se, but a muco-antibody implying that the activity is found to occur with a mucoprotein hapten. This antibody differs from the serum antibody, in that the antibody response following active immunization by parental routes reaches a peak earlier than the serum antibody peak, and disappears while the maximum serum antibody titer exists. Additionally, in contrast to serum antibodies,

this antibody is not cumulative and observed titers may reflect significantly the rate of antibody formation. Quantitative studies support this and infer that the antibody may be found locally.

Associated with this mechanism may be the following unusual fact. It is known that antibodies form locally to cause immunity and fight infection. Work has been done with Trichmonas infection in bovine vaginae to prove that local antibodies form there. This local antibody production is known with other infections. From these facts we may see that:

1. Cervical mucus contains homologous antibodies which are known to have properties to immobilize or agglutinate sperm.
2. There is a possibility that antigenic substances associated with sperm or microorganisms may in certain instances penetrate vaginal mucosa and stimulate local production of antibodies.

Work by Lindahl[21] states the existence of a sperm anti-agglutinin factor in women. This is thought to be formed in the mucus membranes of the fallopian tubes, and it has been demonstrated that anti-agglutinin factor disappears after salpingectomy. It is also found in the growing follicle of the ovary and disappears from follicular fluid after the formation of the corpus luteum. The anti-agglutinin factor is thought to be found in the cervical mucus shortly after ovulation and in

the second trimester of pregnancy. In the normal cycle, titers are thought to be at a peak of 1:2,000 on day fifteen.

3. SPERM ANTIGENICITY

The presence of blood group antigenicity of human sperm was originally described by Landsteiner and Levine in 1926. Their work suggested spermatozoa displayed antigenicity similar to the blood group antigen. The extensive work of Gullbring[11] in 1957 demonstrated segregation of blood group antigens A and B.

Shahani and Southam[35] definitely demonstrated, in 1962, the presence of specific blood group antigens and segregation of these antigens by use of immuno-fluorescent techniques. This work suggested that spermatozoa carry a single ABO blood group antigen on their surface. This would be compatible with the group exhibiting delayed fertility in Behrman's population. The children produced were only of blood group O indicating that A antigens did not cause fertilization and production of group A children.

A consideration in the problem of infertility is that 3% of "male sterile" couples have sperm-agglutinins within the male body fluid system which are similar to the agglutinins developed in the cervical mucus. These antibodies result from stasis which occurs because of occlusion in the vas or epididymis. The sperm may enter the body fluids and the blood stream and infiltrate into the interstitium to cause both local antibody and circulating

antibody production. These auto-antibodies of the male have an action like that of the female antibody.

G. METHODS AND MATERIALS

The women utilized in this study were taken from hetero-specific matings, and in which other causes for infertility had been previously ruled out.

The collection of the samples of cervical mucus was performed by direct aspiration of the specimen with a 10 cc luer-lock syringe to which a small plastic catheter had been attached.

Care was taken to omit any samples which were grossly bloody or exhibited any evidence of infectious organisms.

Determination of the antibody titers was performed by means of a standard doubling dilution titration technique in saline, using 2% washed red cells in a 7 X 50 mm glass test tube. (An equal amount of physiologic saline was added to the cervical mucus specimen which was then mixed, aggitated, and centrifuged. A portion of the supernatent was added to an equal amount of the washed red cells). The end point to be taken was the last tube showing agglutination. There was no control of the hemolysis of the erythrocytes. These tests were performed at room temperature.

The cervical mucus of two patients were utilized in this study. The samples did not show agglutination at any dilution.

H. SUMMARY

1. A possible etiologic factor for previously unexplained fertility initially postulated by Behrman based on an incompatibility of the ABO(H) blood group system has been discussed.

2. The statistical evidence showing a decrease in certain blood group type children has been discussed in light of heterospecific matings and heterospecific pregnancies.

3. The composition, nature, and production of antibodies found in the cervical mucus of normal and infertile individuals has been discussed, this has also included the nature of the antigenicity of sperm.

4. The present concept and knowledge of the secretor status of the population has been summarized.

5. The results have been presented of the laboratory tests of two patients of heterospecific matings with primary infertility. The significance of the results of these tests are at this time obscure.

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