

# IMMUNOLOGICAL PROFILE IN INFERTILE WOMEN

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## Introduction

It is known that during ovulation graffian follicle is formed which ultimately ruptures releasing ovum as well as follicular fluid, whereas ovum is picked up by the fimbrial end of the fallopian tube, the fluid gets absorbed through peritoneal surface. A hypothetical possibility was entertained that in some cases of infertility, the follicular fluid may act as antigen for some reason, thereby leading to production of antibodies in the sera of these women. Such antibodies, in turn, could very well act against the graffian follicle rendering the ovum incapable of fertilisation. The present study was undertaken to explore this possibility of auto-ovum antibodies as one of the causes of infertility in women.

## Material and Methods

The study is based upon the examination of 75 cases. Of these, there were 25 cases each of primary infertility (Group I) and of secondary infertility (Group II). The remaining cases were derived from normal controls (Group III). Relevant clinical, biochemical and pathological investigations were carried out in each

case to establish the diagnosis of infertility and to rule out any obvious cause of infertility.

## Collection of Materials

Five types of ovarian fluids were collected from the patients undergoing hysterectomy or laparotomy for various reasons. These fluids labelled as A, B, C, D and E as follows:

- |                                    |   |
|------------------------------------|---|
| 1. Follicular cyst fluid           | A |
| 2. Corpus luteal cyst fluid        | B |
| 3. Benign serous cyst fluid        | C |
| 4. Benign serous cystadenoma fluid | D |
| 5. Pseudomucinous cystadenoma      | E |

In cases of follicular cyst and corpus luteal cysts the diagnosis was clinical, whereas in the remaining cysts the diagnosis was established through histopathological examination of ovaries. The various fluids were collected under aseptic condition from intact cyst with sterile syringes so as to avoid contamination with blood or other body fluids. These were then stored at 4°C till used. These fluids were used as antigens and their antigenicity were determined through animal experimentation performed on rabbits.

## Testing of Antigenicity

Five mature healthy male rabbits were selected and were kept in laboratory for

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15 days. The rabbits were provided with rabbit feed (Hindustan Lever Ltd.) and water *al libitum*. These rabbits were labelled as A, B, C, D, E according to the fluids and the fluids were injected in the corresponding rabbits. Before administering the ovarian fluids, 3 ml. of blood was collected from ear vein of each rabbit and allowed to clot and serum so separated were labelled and stored at 4°C till used. Half ml of each type of ovarian fluid was mixed with half ml of Freund's complete adjuvant (Difco) in separate sterile syringes. Injection was given at two separate sites:

1. Intradermal in anterior abdominal wall of rabbit.
2. Foot pad of rabbit.

Injections were repeated after a week and blood samples were collected from ear vein on 10th, 15th and 20th day of administration of the ovarian fluid till the sera gave a positive reaction for antibodies by Agar-gel diffusion technique. At this stage, 10 ml blood was collected from the ear vein of the rabbits, sera were separated and stored at 4°C after labelling them as anti-A, anti-B, anti-C, anti-D and anti-E corresponding to the labelling of fluids of ovary. These sera were used to serve as positive controls in the immunological tests employed.

For the purpose of detecting antibodies against the various fluids of the ovary, 3 ml of blood from antecubital vein was collected from the patient, allowed to clot and serum was separated after centrifugation. The sera were then stored at 4°C after proper labelling till tested. These sera were tested against the pooled ovarian fluids by the following techniques:

1. Agar-gel diffusion technique (Ouchterlony, 1918).

2. Counter-current immuno-electrophoresis (Psendorfer *et al* 1970).

#### Observations

In the present study cases selected had no systemic illness and pathology and husband's semen was normal in amount and viscosity, count, motility and morphology. Cervical and vaginal smears were taken on various days of cycle and showed that ovulation occurred. Endometrium showed secretory phase in all cases. No evidence of any disease was found on histopathological examination.

#### Observations of Immunological Tests

Sera collected from blood which was taken from labelled rabbits before starting administration of ovarian fluids were tested against corresponding fluids by Agar gel diffusion. All fluids gave a negative reaction.

Now sera collected from blood samples of rabbits on 10th, 15th and 20th day were tested with corresponding fluids. With all types of fluids precipitation line was observed against corresponding fluid. These reactions came positive with sera taken on 15th day of first injection in all fluids except corpus luteal fluid in which ppt. line was observed on 20th day of first injection. Table I shows period of appearance of antibodies in rabbits.

Now various ovarian fluids were tested against human sera derived from cases of infertility as well as normal fertile couples by both techniques.

#### Observations with agar-gel diffusion technique

##### (A) Follicular cyst fluid

This fluid as an antigen gave 8 positive precipitation lines in group I cases, one positive reaction with group II and all

TABLE I  
Relation of Time Period with Antibody Formation

Type of ovarian fluid	Time period from first day of injection			
	0 days	10th day	15th day	20th day
A	—	—	+	+
B	—	—	—	+
C	—	—	+	+
D	—	—	+	+
E	—	—	+	+

A: Follicular Cyst fluid

B: Corpus luteal cyst fluid

C: Benign serous cyst fluid

D: Benign serous cystadenoma fluid

E: Pseudomucinous cystadenoma fluid.

negative reaction with control group i.e. group III.

(B) *Corpus Luteal fluid*

As an antigen gave no positive reactions with any of the 3 groups under study except control precipitation line by using Antisera B.

(C) *Benign serous cyst fluid*

As an antigen, 3 cases of group I gave positive reactions, 1 case of group II showed positive precipitation line, while no case from control group (Group III) gave positive reaction.

(D) *Benign serous cystadenoma fluid*

As an antigen no case from either of 3 groups gave positive reactions except for control precipitation line formed by using Antisera D.

(E) *Pseudomucinous cystadenoma fluid*

Having this fluid which is totally a pathological cyst, no positive reaction could be noticed in any of the 3 groups.

A peculiar reaction which was observed with all types of antisera of fluids was the precipitation lines formed between the specific antisera and patients' antisera. To confirm whether this precipitation line

TABLE II  
Observations of Agargel diffusion Technique

Ovarian fluids (Antigen)	Group I		Group II		Group III	
	Number of cases		Number of cases		Number of cases	
	Positive Reaction	Negative Reaction	Positive Reaction	Negative Reaction	Positive Reaction	Negative Reaction
A	8	17	1	24	0	25
B	0	25	0	25	0	25
C	3	22	1	24	0	25
D	0	25	0	25	0	25
E	0	25	0	25	0	25

was due to spilling or overflow, corresponding antisera were put in the central holes and all surrounding holes were charged with patients' and one hole with the corresponding fluid, which acted as control. A continuous encircling line between all holes which was faint at places as compared to control was observed, which indicated reaction between rabbit antisera and sera of patients with various groups.

#### Observations with counter-current immuno-electrophoresis

The observations derived from this technique were similar to the ones derived from agar-gel technique, and no differences in positive/negative reaction were seen, except that the precipitation lines seen between rabbit's antisera and patient's sera by gel diffusion technique was not observed with this technique.

The results derived from these observations are shown in Table III.

Moreover, each type of rabbit antisera were tested against all 5 fluids, to see cross reactivity of antigens and it was found that rabbit antisera A reacted with follicular cyst and serous cyst fluid, but did not react with the other fluids. Similarly, antisera C also reacted with follicular cyst and serous cyst fluids. Antisera B, D and E gave only positive reaction against corresponding fluids.

#### Discussion

Animal experiments conducted during this study, clearly establish the antigenic nature of both normal and abnormal (Pathological) fluids obtained from various types of ovarian cysts. Amongst the various ovarian fluids, follicular cyst fluid at least is known to be antigenic in nature. (Shivers *et al* 1964; Desjardin 1966; Harve *et al* 1968 and Jeffcoate 1976). Observations in the present study, however, clearly establish that besides follicular cyst fluid, all other types of

TABLE III  
Observations With Immuno-electrophoresis

Ovarian fluid	Group I		Group II		Group III	
	No. of cases		No. of cases		No. of cases	
	Positive Reaction	Negative Reaction	Positive Reaction	Negative Reaction	Positive Reaction	Negative Reaction
A	8	17	1	24	0	25
B	0	25	0	25	0	25
C	3	22	1	24	0	25
D	0	25	0	25	0	25
E	0	25	0	25	0	25

Further, on comparing the positive cases of Group I and Group II against antigens A and C, it was noticed that 3 positive reactions with antigen C in group I had also positive reactions with antigen A. In Group II cases showing positive reaction with antigen A also showed positive reaction with antigen C.

ovarian fluids tested were also antigenic as proved by precipitation line formed with corresponding antisera.

However, a reaction was observed between human female sera and antisera prepared from rabbit. This cross reactivity may be on account of the same

antigen being common in both ovarian fluids and human sera.

When rabbit antisera was tested against the five types of fluids, it was found that rabbit antisera A reacted not only with follicular cyst but also with serous cyst fluid. Similarly, antisera C also reacted with follicular cyst and serous cyst fluid. Antisera B, D and E gave positive reaction against corresponding fluids only. This may be on account of the fact that fluids in these 2 types of cysts do not differ in qualities. Hence, positive reaction obtained with antigen A and C might be due to similar antigenic nature but even if it is so, then why all cases which gave positive reaction against antigen A did not reveal similar reaction with antigen C, needs thorough probing.

In the present study, comparative evaluation of 2 serological techniques was made since electrophoretic technique is considered to be far more sensitive than agar gel diffusion technique while no difference in sensitivity was observed, the former technique proved to be more specific in the detection of the antigen antibody reactions as the cross reactivity observed by Agar gel diffusion technique was not observed with this technique.

Analysis of the observations in regard to presence of specific antibody against ovarian fluids has revealed that normally no antibody is found in the sera of infertile women against any of the fluids. Negative reaction with pathological fluids—serous cyst adenoma and pseudomucinous cystadenoma and one physiological fluid i.e. corpus luteal fluid, in all cases indicate absence of antibodies against them in the body; control group gave negative reaction with all types of antigens indicating that this type of antigen antibody reaction is not a normal phenomenon.

#### *Summary and Conclusion*

From the above study done in 75 cases, we can conclude that:

1. All type of ovarian fluids are antigenic in nature.
2. Follicular cyst fluid and serous cyst fluids are cross antigenic and might contain same antigens with some quantitative differences.
3. Out of 25 cases of primary infertility, 8 showed positive precipitation line with Antigen A.
4. In 25 cases of Group I, only 3 showed positive reaction with Antigen C.
5. One out of 25 cases of Group II gave positive precipitation line with Antigens A and C.
6. All 25 cases of group III, i.e. Control fertile group gave negative reaction.
7. Antibodies formed against all types of ovarian fluids' antigens in rabbits were not specific against these ovarian fluids and also cross reacted with some antigens of patient's sera.
8. Corpus luteal fluid and two pathological fluids all gave negative reaction in all groups of cases.

From the above discussion, it could be concluded that hypothesis of auto-ovum antibodies formed by auto-antigenicity of follicular fluid bears some weight and might play role in few cases of primary infertility group. These are not proved to be a cause in secondary infertility group in which only 1 case showed positive reaction i.e. 4% cases. In primary infertility cases, 32% gave positive precipitation line for auto-ovum antibodies. When compared to zero per cent positive reaction in control group, it is significant. Hence auto-ovum antibodies in a woman's sera play a role and might kill or devitalize ovum so that it is not capable of fertilization and cause infertility. More

detailed clinical studies are in progress and shall be reported elsewhere.

References

1. Desjardin, C., Kirton, K. R. and Hafs, H. D.: *Some Chemical J. Reprod. Fertil*, 11: 237, 1966.
2. Herve, R., Sergent, P. and Rolley M.: *Presses Med.* 76: 2421, 1968.
3. Jeffcoate, T. N. A.: *W.H.O. Bibliography*, October 1976, Supplement, No. 9.
4. Ouchterlony, O.: *Diffusion in gel method for immunological analysis. Progr. Allergy*, 5: 1, 1918.
5. Psendorfer, et al—*Klinwache*, 48: 58-59, 1970 (quoted by W.H.O. Vol. 42, 1970).
6. Shivers, C. A. Metz, C. B. and Lutwan Mann, C.: *J. Reprod. Fertil.*, 8: 115, 1964.