PREPARATION OF IMMUNOGEN CAPABLE OF AGGLUTINIZING SPERMS OF ALBINO RABBITS

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Introduction

The advent of immunological concept and antigen antibody reaction brought a new horizon within the scope of investigators of antifertility methods. The antigenicity of semen and testicular material was first demonstrated by Metchnikoff (1899) and Landsteiner (1899). Different types of antibodies have been detected in some females by Tyler (1941). Rumke and Hellinga (1959) demonstrated similar antibodies in the sera of infertile males. Graham and Graham (1959) prepared antibodies against sperm by injecting the testicular material in guinea-pigs and observed that fertility in female guinea-pig was not effected. Tsunda and Chang (1976) demonstrated suppression of reproduction in rat and mice through prepared antibodies. The present study was planned to prepare antibodies against sperm and seminal fluid antigen in rabbits. The effect of prepared antibody was studied to see the ability of antibodies to agglutinate the sperm.

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Material and Methods

The study was made on male albino rabbits weighing 1-2 kg. fed on soaked gram and water ad-lib. The rabbits were divided in three groups.

Group A: The male albino rabbits for preparation of immunogen.

Group B: Control—20 male rabbits.

Group C: Twenty male rabbits to study the effect of prepared immunogen on the sperms *in vitro*.

Group A: Extracts of cauda epididymis of rabbits were centrifuged (3000 RPM x 15 minutes at 20 degree C). The supernatant fluid constituting seminal plasma was decanted. Sperm pellets were washed twice with sperm diluent (Schnieder's *et al*, 1975).

Schnieder's diluent

Compound	Molarity	Volume
NaCl	0.154M	100 ml.
KCl	0.154M	6 ml.
Mg So_4	0.154M	1 ml.
Phosphate buffer (Ph 7.5)	0.150M	5 ml.

The sperm pellets were washed and resuspended to the original volume in sperm diluent. This preparation was centrifuged (3000 RPM x 15 minutes at 20

degree C) and equal volume of Freund's adjuvant was added to the above preparation. The preparation was used as immunogen.

Five male albino rabbits were injected with 1 cc. of immunogen after every 10th day for 5 weeks. Six days after the last injection blood samples were collected from all the rabbits and serum was separated by centrifuging the blood (3000 RPM x 15 minutes at 20 degree C). The separated serum was used as antisera with an assumption that serum contained antibodies against the sperms.

Group B: Sera from the standard group was collected in the above manner. Each specimen was explored under 20 fields of microscope (43 X). Motility of sperms and type of agglutination (Head to head and head to tail) were observed. Wherever agglutination was found it was labelled as 25%, 50%, 80% or 100% depending upon amount of agglutinated sperms after matching the sperm pellet obtained from either group with prepared antisera.

Results

Group A: It was expected that immunogen had provoked antibody formation in the sera.

Group B: When antisera was matched with sperm pellet *in vitro*, no agglutination was found. Motility, shape and size of sperm remained normal when examined under microscope (43 x) even after 3 hours.

Group C: Sperm pellets of group C (immunogen injected) were matched

TABLE I

Report of Antisera on Sperms in Albino Rabbits (Agglutination and Durat-ion Relationship)

No.	Start (sec.)	50% (mts)	80% (mts)	90% (mts)	100% (mts)
1.	20	2	4	8	10
2.	30	5	4.5	8.5	9.5
3.	15	1	6	8	11
4.	30	1	8	10	12
5.	30	1	8	9	14
6.	30	2	7	10	14.5
7.	60	2	6	10.5	14
8.	30	3	4.5	10	15
9.	150	1	8	15	18
10.	30	3	7	8	13
11.	30	5	12	23	28
12.	60	3	13	20	35.5
13.	30	4	15	25	30
14.	60	2	10	24	28
15.	30	3	8	20	30
16.	30	10	5	16	36
17.	40	2	6	15	27.5
18.	30	4	7	10	26
Total 735		54	139	250	372
Average time 40.8 Sec.		3 m'	7.72 mts.	13.88 mts.	20.66 mts.

with antisera *in vitro* and were observed under microscope. In the 1st rabbit, agglutination started within 15-20 seconds. Agglutination was massive (about 80%). The motility of sperms was gradually reduced and after 10 minutes almost 100% agglutination was found. Both head to head and head to tail agglutination was found in all the fields (Fig. 1). Agglutination power continued to decrease alongwith the length of time. In the later specimens agglutination started after ½ minute and agglutination was about 14.5%. At the end of the study the agglutination was found to be 100% but took about 2 minutes (Tables I and II; Graphs 1 and 2).

TABLE II

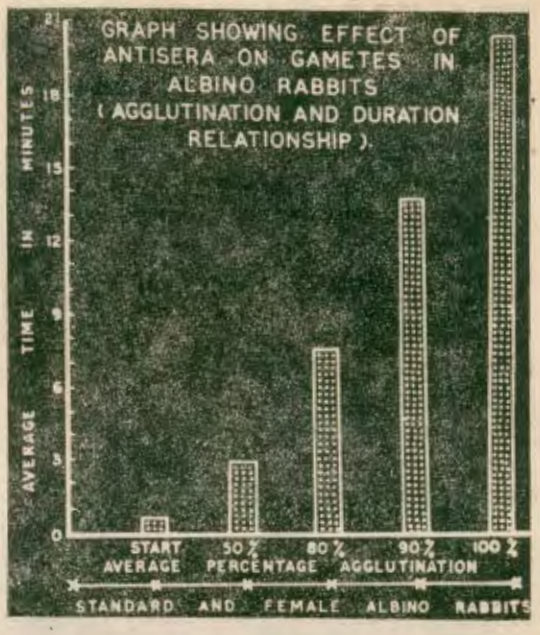
Report of Antisera on Sperms in Albino Rabbits

S. No.	Starting (Secs)	100% (mints)
1.	15	9.5
2.	15	10
3.	20	11
4.	30	12
5.	30	13
6.	30	14
7.	30	14
8.	30	14.5
9.	30	15
10.	30	18
11.	30	28
12.	30	28
13.	30	30
14.	60	30
15.	60	35.5
16.	75	36
17.	120	36
18.	120	37

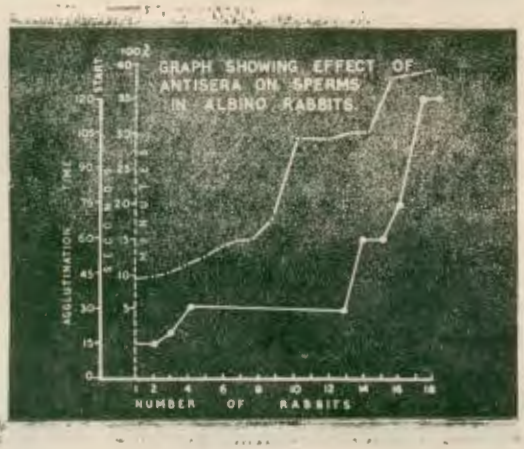
Discussion

The reproduction and family planning is basic desire and need of human beings and the phenomenon is as old as age of mankind itself. It is very strange that our ancestors considered talk of reproductive system as a subject not to be talked till 19th century. The discussion or investigations of this fundamental functions of origin of life was considered as a sin and literature was labelled as obscene. Marshall's treaties made a break through in the study of reproduction and is still accepted as an authority on the subject. Tyler (1941), Rao and Sadri (1959) demonstrated an antigenic coat on the surface of sperm. Wilson (1954), Rao and Sadri (1959), Rumke and Hellinga (1959), Franklin and Dukes (1968), Rao and Rangnekar, Glass and Vaidya (1970), Vaidya and Glass (1971), demonstrated antibodies against sperm in sera of women with unexplained infertility. Nath *et al* (1976), Kumar *et al* (1976) and Talwar G. P. were able to suppress fertility by Pr-B-HcG-T-T. Many investigators have demonstrated presence of such antibodies and postulated that these have the capability to agglutinate sperms in experimental animals. In the present study, antisera had no effect on sperm pellets obtained from standard group. While agglutination was observed when sperm pellets of immunogen treated rabbits were matched with antisera. This leads to the conclusion that antisera contained antibodies against sperms. Metchnikoff (1899) has also demonstrated that production of antibodies was possible and was capable of agglutinating and immobilizing sperms. High antigenicity of seminal fluid had been well established by many investigators. Atleast 6 distinct antigens had been demonstrated. Quinlivan (1966), Weil and Finkler (1958), Weil and Rodenberg (1962) suggested that this antigenicity was due to seminal plasma or coat on the spermatozoa. Hekman and Rumke (1968) identified it as an iron binding protein called Lactoferin. While Parish and Ward (1968)

deferred the above postulates. Result of the present study show that antibodies were present in sera (obtained from animals injectted with immunogen). The agglutination started in 15-20 seconds with an average of 41 seconds in all the specimen examined and reached to the level of 50% within 3-4 minutes and 100% agglutination started in 2 minutes and was completed in 20 to 36 minutes (Table I), when the agglutination was compared with the duration or stability of sera it was observed that earlier specimen agglutinated as early as 15 seconds, while in the later specimens agglutination started in 120 seconds. 100% agglutination in earlier specimen was found in 9.5 minutes. On the basis of the results of present study it is postulated that immunogen provoked antibody formation in the sera of injected animals. This sera had the capability to agglutinate sperms, but perhaps antibody titre of sera continued to fall with longevity of time (graphs 1, 2). Many workers have suggested that B-globulin fraction was responsible for such reaction. Schnieder *et al* (1975) have also demonstrated that agglutination of heterologous sperms in rabbits occurs with prepared antibodies due to the change in the membranous



permeability of the sperm. Ackerman and Gonzalez (1969) and Goldberg (1972) tried to explain the above antigen antibody reaction on the basis of enzymatic reaction interfering with oxygen consumption, membrane permeability, enzymatic and metabolic reactions were not studied although the possibilities of such changes cannot be overlooked. Our results show that only agglutination occurred, no cytolysis was observed in any specimen as described by Wilson (1956).



Conclusion

1. Sperms collected from cauda epididymis alongwith Freund's adjuvant when injected in rabbits acted as immunogen and provoked antibody formation which acted as antisera.

2. The above antisera agglutinated sperms collected from cauda epididymis. Agglutination of head to tail and head to head type was found.

3. The agglutination capacity of sera continued to fall with longuity of period.

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See Figs. on Art Paper VI