5. DISCUSSION

5.1. Reaction with normal serum

The normal sera of rabbits which were immunized with epididymal spermatozoa and ejaculated washed spermatozoa of goats gave negative reactions in sperm agglutination and immobilization tests.

Edwards (1960) observed complement fixing activity in the normal serum of rabbits against epididymal and ejaculated washed spermatozoa of the same species. Passive cutaneous anaphylaxis, immobilization and precipitation reactions, however, failed to demonstrate antibody activity against spermatozoa.

Spooner (1964) observed antibody activity in guinea-pig which reacted with spermatozoal acrosome and testicular cells respectively of the same animal. Johnson (1968) observed a complement fixing antibody to the spermatozoal acrosome in the normal guinea-pig serum.

The occurrence of sperm agglutination with normal saline has been observed by various workers in different species (Kiddy et al., 1959; Menge et al., 1962; Edwards, 1960; Prasad and Nair, 1973).

Antibody activity observed by Spooner (1964) was due to formation of autoantibody against guinea-pig spermatozoa and
testicular cells. The formation of autoantibodies in unimmunized animals has been considered to be due to break down of testicular barrier which normally acts as a protective mechanism.

In the present study, the sperm agglutination, immobilization as well as precipitation reaction in gel showed the absence of antibodies against goat spermatozoa in the normal sera of rabbits. Sperm agglomeration was seen with certain semen samples and normal saline, but such samples were not used.

Negative reactions with the normal sera of different rabbits led to the conclusion that naturally occurring antibodies against ejaculated and epididymal spermatozoa of goats were absent.

5.2. Anaphylactic reactions in rabbits

The death of rabbit No.109 under experimental immunization with ejaculated washed spermatozoa was due to hypersensitivity of immediate type. The reactions exhibited were typical of anaphylaxis.

In a previous study, Prasad (1969) observed that rabbits are very prone to anaphylactic reaction due to intravenous injections of bovine semen. Anaphylaxis resulting from sensitivity of highly antigenic substances causes release of histamine, serotonin and bradykinin from sensitized tissue (Geiger and Alpers, 1958; Chase, 1965).

The spermatozoa being highly antigenic material induced immediate type of hypersensitivity in the rabbit No.105. As a precautionary measure, the subcutaneous and intradermal routes were used, which proved to be efficient in avoiding hypersensitivity
reactions in rabbits that were used for heteroimmunization against various antigens of male reproductive tract of goats.

5.3. Role of adjuvant in increasing immunological response

The use of water-in-oil adjuvant mixture considerably increased the potency of immune sera. In the rabbits, the use of Freund's complete adjuvant promoted a heightened immunological response which was indicated by gradual elevation of the titres of immune sera in rabbits 124 and 132 at different intervals during the course of immunization. A subcutaneous granuloma with sharply demarcated rubbery firm mass developed at the site of injection.

The addition of mycobacterial wax D (glycolipids and peptido-glycolipids) results in a pronounced increase in macrophages, locally or at the remote site (Askonas and White, 1956). The water-in-oil emulsion serves to delay absorption and elimination of the antigens from the site of injection (Humphrey and White, 1970).

Goyal et al. (1968) used six percent alum solution as an adjuvant and obtained 82.51 percent gamma-globulin in the hyperimmunized goats against 38.58 percent before immunization with buck semen. The increased levels of sperm agglutinating and sperm immobilizing antibodies in IS 124 and IS 132 were evidently due to prolonged absorptive action of antigen–adjuvant mixture. The subcutaneous granulomatous tissue observed at the site of injections appeared to increase considerably the cellular response in the experimental animals. This resulted in the production of potent antibodies.
5.4. Antibodies against spermatozoa

5.4.1. Agglutinating antibodies against ejaculated washed spermatozoa of bucks

The potency of immune sera from rabbits No.124 and 132 was judged by the activity of sperm agglutinating antibodies which gave +++ reaction upto 1/64 and 1/256 dilutions respectively for the two sera. These reactions were obtained at the 13th week of immunizations (Table I).

A gradual increase took place in the activity of sperm agglutinating antibodies in IS 124 and 132 as evidenced in figure I. The antibodies against washed ejaculated spermatozoa of goats continued to rise and reached upto 1/128 and 1/256 respectively. A decline was observed after 90th and 100th days of immunization.

The highly antigenic nature of semen of various animals has been demonstrated by a number of experimenters (Landsteiner, 1900; Henle et al., 1938; Weil and Finkler, 1959; Rao et al., 1959 and Mittal et al., 1965). Edwards (1960) observed that homologous semen was weakly antigenic in rabbits.

Pandey (1966) also observed a rising tendency of antibodies in goats immunized against buck semen. The titres of antisera ranged from 1:40 to 1:320.

Goel et al. (1968) tested the hyperimmunity of goats immunized against buck semen and against bull semen using indirect haemagglutination.

The trend of rise in the activity of spermagglutinating antibodies in immune sera 124 and 132 and subsequent decline (Fig. I)
has been similar to that observed by Edwards (1960) against rabbit semen. Kiddy et al. (1959) and Mange (1967, 1969) obtained a similar response in heifers.

The immune sera against antigens of ejaculated washed spermatozoa of bucks could specifically detect the sperm-agglutinating antibodies that were produced in IS 124 and 132.

5.4.2. Agglutinating antibodies against epididymal spermatozoa

The sperm agglutinating antibodies against epididymal spermatozoa were obtained from rabbits No. 113 and 136. The antigenic response in both the rabbits was relatively weak as compared to that against ejaculated washed spermatozoa. The titres of the antisera against epididymal sperms rose to 1/64 and 1/128 respectively (Table II).

Several attempts have been made to induce antibodies against epididymal spermatozoa in rabbits (Henle, 1938) and in mice (Snell and Poucher, 1943). Washed epididymal spermatozoa elicited isoantibodies in rabbits. The antigens detected either originated in the testes or epididymis or both. The antigens which originated in epididymis might become coated on spermatozoa. Report of Weil (1962) suggested that epididymal sperms could absorb the antigens of seminal plasma.

The increased antigenicity of the ejaculated washed spermatozoa as compared to epididymal spermatozoa of bucks in the present work could be due to some of the sperm coating antigens which are acquired during their passage onwards from epididymis.
Some of these might constitute seminal plasma antigens which strongly bind on the spermatozoa and are not removed even after several washings.

It has also been suggested that sub-surface location of some of the sperm specific antigens and autoantigens protect the individual from being immunized. Evidently the poor antigenic nature of epididymal sperms could be due to this protective mechanism, thus making the antigens unavailable for combination with antibody in intact cell. This is supported by the findings of Rao et al. (1959) that repeated freezing and thawing released certain intracellular antigens. Thus the low titres of immune sera 113 and 136 could also be due to poor response in the rabbits as well as weaker antigenicity of the epididymal spermatozoa of goats.

5.4.3. Agglutinating antibodies in immune sera produced against testes homogenate

The response of rabbits No. 108 and 137 that were immunized with testes homogenate of goats has been shown in Table III. The hyperimmune sera against testes homogenate were tested with epididymal spermatozoa presuming that the epididymal sperms possessed some common antigenic components.

The immune sera from rabbit No. 108 reached a titre of 1/64 after 12th week and that of IS 137 went up to 1/128 after 14th week. The intensity of spermagglutinating antibodies varied in both these sera. Good reaction (+++) was observed in the immune serum 108 up to 1/8th dilution. The IS 137 showed +++ reaction
upto 1/32 dilution. These titres were seen upto 14th week of immunizations. Thus the IS 108 was less potent than IS 137.

Varying degrees of response have been obtained by different workers against antigens of testes homogenate. Katsh (1959) in guinea-pigs, Menge (1967, 1969 and 1970) in rabbits and heifers. Menge (1970) studied the antigenicity of primary and secondary spermatocytes of rabbits and heifers. Menge (1969) observed a titre of 1/16 to 1/32 in heifers immunized with immature testes. A titre of 1/32 to 1/64 was obtained by Menge (1970) in rabbits immunized against primary and secondary spermatocytes.

The immune sera from rabbit No. 108 appeared to be less potent than IS 137. The variation could be due to differences in the immune response of the rabbits against antigenic components of testes homogenate. The results of earlier investigators on the antigenicity of rabbit and bull semen appeared to show a similar trend to what was obtained with buck testes homogenate in the present study. These results indicated that antigenic components of testes homogenate of mature animals was weaker than that of ejaculated spermatozoa and seminal plasma.

5.4.4. Pattern of sperm agglutination

Treatment of semen samples with low dilutions of immune sera gave rise to tail-tail agglutination. The intermediate dilutions resulted in mixed agglutination in which aggregates of heads and tails were formed. High dilutions of immune sera caused head-head agglutination. The normal sera of rabbits did not show agglutination. Occasionally some of the samples treated with normal
sers showed agglomeration of heads which could be distinguished 
from agglutination with immune serum. The epididymal spermatozoa 
of bucks showed clumping of heads and tails referred to as mixed 
agglutination. The different dilutions resulted in varying degrees 
of intensity of clumping.

A consistent pattern of agglutination has been 
observed by various workers. Henle et al. (1938) demonstrated 
the head and tail specific antigens as well as an antigen common 
to both head and tail. Kiddy et al. (1959), Edwards (1960) and 
Krieg (1970) also observed tail-tail agglutination with low dilutions 
and head-tail (mixed) agglutination with high dilutions of immune 
serum. Beck et al. (1962) and Hansen (1972) demonstrated the tail 
fluorescence of guinea-pig, bull and rabbit spermatozoa.

The results presented here showed that tail-tail 
agglutination was observed only on treatment of semen with immune 
sers. This agrees with the findings of Henle et al. (1938), 
Kiddy et al. (1959), Edwards (1960), Menge et al. (1962) and Krieg 
(1970). The report of Beck et al. (1962) that tail fluorescence of 
guinea-pig spermatozoa was specifically due to reaction with immune 
serum, further supported the present study. Similarly the 
ocurrence of head-head and mixed agglutination with intermediate 
dilutions of immune sera have been noted by all these workers. 
However the work of Beck et al. (1962) demonstrated conclusively 
that the acrosomal fluorescence of guinea-pig sperm was due to 
reaction with normal serum.

The normal sera of rabbits has been observed to cause 
head-head agglutination of buck spermatozoa, but the intensity of
reaction was very weak, therefore it could be distinguished from head-head agglutination due to reaction with higher dilutions of immune sera. The occurrence of head-head agglutination with normal sera has also been noted by Kiddy et al. (1959), Menge et al. (1962) and Bedford (1970) with rabbit semen and Prasad and Nair (1973) with bull semen.

These results indicated that the different patterns of agglutination reactions observed were due to the action of antibodies against spermatozoa. The tail-tail pattern which is specifically caused by low dilutions of immune sera might be due to some of the antigens that were located on the tail portion of spermatozoa. The head-head agglutination caused by higher dilution could be due to reaction of antigens located on the surface of sperm heads whereas the mixed agglutination might be produced as a combined action of those antigens that were shared both by heads and tails. Thus the sperm agglutinating antigens of buck spermatozoa are distributed on different parts of the cell. Further these reactions could also be produced due to combined action of certain sub-surface antigens that might have been produced as a result of immunization done with repeatedly frozen and thawed samples of sperms.

5.4.5. Immobilising antibodies against ejaculated washed spermatozoa of goats

The activity of sperm immobilising antibodies in the sera of rabbits 124 and 132 was tested with an idea of detecting anti-spermatozoal antibodies that could specifically inactivate sperm in fresh semen and impair fertilizing capacity.
The sperm immobilizing activity of immune sera 124 and 132 was tested using fresh semen from three bucks. The spermatozoa of different bucks differed in the intensity of motility on treatment with immune sera. The immobilising antibodies of these two sera appeared to react differently and produced immobilization of the sperms in fresh semen of bucks No.1, 10 and 11 respectively (Tables IV, V and VI).

Lower dilutions of immune sera 124 (1/2 to 1/4) caused immediate inhibition of wave like motility of semen from bucks No.1, 10 and 11. Similarly IS 132 also caused immediate arrest of wave like motion of semen from bucks No.1, 10 and 11 on treatment with 1/2 to 1/8 dilutions.

Higher dilutions of both the immune sera appeared to produce less profound effect on the motility of semen of bucks (Tables IV, V and VI). The treatment with higher dilutions invariably resulted in formation of clumps of different sizes. In these clumps a number of sperms were seen possessing different kinds of motion, viz., vibratory, side to side and limping. However progressive motility was generally absent except a few sperms of buck No.11 on treatment with 1/128 dilution of IS 124 (Table IV). Higher dilutions of the immune sera took longer time to produce the immobilizing effect than lower dilutions.

The intensity of immobilizing antibodies differed with different dilutions of immune sera and semen from bucks. The lower dilutions of immune serum 124 produced higher degree of immobilization (++++) as compared to higher dilutions (1/64 to 1/128) which had lower activity (Tables IV and V).
The motility of active spermatozoa is said to be due to rhythmic contractions of alpha, beta and gamma fibrils in the mid portion of tail (Nelson, 1967). The anatomical architecture of these fibrils provides a suitable mechanism for wave like motion.

The report of Back et al. (1962) that induced antibodies were produced against tail antigens supported the view that agglutinating and immobilizing antibodies might impair active rhythmic contractions of different fibrils of the tail of motile spermatozoa. In the present study, it has been demonstrated that immobilising antibodies present in IS 124 and 132 affected the wave like motion of normal sperms.

Mittal et al. (1965) concluded that metabolic activity of immune serum treated with spermatozoa of cattle was reduced due to death of sperm cells. Prasad and Nair (1973) also noted a similar effect with bovine semen.

In the study presented here, the death of spermatozoa followed immobilization. Large number of treated sperms continued to remain viable with vibratory, side to side or limping movement.

The titre of sperm immobilizing antibodies in both the sera reached 1/1024 (Fig. II). The lower intensity of immobilization with higher dilutions might be due to suboptimal concentration of antibodies required to completely inhibit active motion of sperms.

The samples of semen of buck No.1 on treatment with IS 132 showed vibratory movement. This was in contrast to complete inhibition with IS 124 (Table IV). The differences in the immobilizing pattern could be due to differences in the potency of immune sera. The differences in the intensity of reactions
observed with different semen samples and immune sera could also
be due to dilution effect of semen samples.

The immediate cessation of wave like motion of freshly
ejaculated spermatozoa on treatment with lower dilutions could also
be due to higher potency of the antibodies. In both lower and
higher dilutions the motility of good quality semen of bucks was
affected severely.

The mechanism of action of antibodies affecting the
motility has not been clearly understood so far. In paramecium
immobilization occurs due to agglutination of cilia. The cilia
possess antigenic substances which induce immobilizing antibodies.
The ciliary antigens of paramecium have been characterized (Preer,
1959).

Since the sera IS 124 and 132 were produced with
repeatedly washed, frozen and thawed samples of spermatozoa, it
could be most likely that a number of sub-surface sperm antigens,
including, the immobilizing ones might have been released resulting
in production of corresponding antibodies of immobilizing type.

The sperm immobilization test has been considered to be
a better test than sperm agglutination test, and is of diagnostic
value since the results of this correlated with cases of
unexplained infertility (Eyquem, 1969).

The results on the sperm immobilization of treated
samples of semen showed that the sperm immobilizing antibodies
acted specifically on the active motile spermatozoa and caused the
cessation of normal wave like motion which might result in
impairment of fertilizing capacity.
These results are compatible with those of Isojima et al. (1968) and Isojima (1969) in as much that sperm immobilization test is more specific than agglutination in detecting antibodies against spermatozoa which impaired fertilizing ability.

5.5. Effect of immune serum on viability of spermatozoa

A decrease in the viability of samples treated with different dilutions of immune sera was noted at different intervals of time (10 to 30 minutes). The viability percent of samples treated with dilutions of immune sera IS 124 and NS 124 ranged from 27.00 to 38.70 and from 60.00 to 66.35. The live counts of untreated samples tested at different intervals ranged from 63.23 to 68.35.

A similar trend was seen on treatment of different dilutions of immune serum 132. The viability percent ranged from 24.60 to 38.51 with 1/2 to 1/16 dilutions tested at different intervals of time (Tables VII and VIII). The values for normal sera and untreated controls ranged from 60.25 to 68.65 and 67.37 to 72.35.

Mittal et al. (1965) studied the metabolic effect of spermatozoa on samples of bovine semen treated with immune sera prepared against bull semen. Their studies indicated a drastic reduction in oxygen uptake, fructose consumption and lactic acid production. A severe decrease in the live count of the treated sperm was noted which was due to the death of cells.

Kiddy et al. (1959) observed that lower dilutions of immune sera against bovine spermatozoa caused fertilization failures. They observed lower values of embryo survival in immunized rabbits. Similar results were observed by Hange et al. (1962) in rabbits.
In a previous study Prasad and Nair (1973) observed a significant effect of immune sera on the percentage death of spermatozoa of bulls treated for increasing duration of time. The low dilutions of immune sera caused higher mortality. Normal sera were found to have no effect on the viability of spermatozoa.

The decrease in the viability of spermatozoa of goats treated with different dilutions of immune sera was due to the death of cells as seen by staining reaction with eosin-nigrosin. The death was caused due to the action of antibodies against buck spermatozoa. This was also evident from the results of untreated samples and the samples treated with normal sera (Tables VII and VIII). The mortality of treated sperms ranged from 61.30 to 73.00 percent with IS 124. The mortality of sperm samples treated with IS 132 ranged from 61.49 to 75.40. In contrast to this, the mortality of samples treated with normal sera ranged from 33.65 to 40.00 and 31.35 to 39.75. The values for untreated samples were 31.65 to 36.77 and 27.65 to 32.63 respectively. The decline of viability in the untreated samples was very small (Tables VII and VIII). A majority of population of sperm cells appeared to be affected within ten minutes of treatment. Incubation for higher durations up to 30 minutes caused a small decrease in the live percentage. The results on viability of spermatozoa treated with immune sera showed that 25.00 to 38.70 percent of treated sperms remained alive.

Thus these results showed that antibodies against buck spermatozoa caused death of majority of sperm cells. Approximately one-third of the treated sperms escaped the lethal action of antibodies and remained alive even after 30 minutes of treatment.
These may be available for fertilization and this could be one reason why antisera have been ineffective in inducing infertility.

5.6. Studies on antigenic composition of testes and male reproductive secretions of goat

The male reproductive tract consists of testes and accessory glands which are rich in several antigens. For precise understanding the specific role of the various antigenic materials, it is important to study and delineate the antigens contributed by each one of the accessory organs of reproduction. This is likely to help in exploring the level of antigenicity and the nature of these antigens.

5.6.1. Antigens of testes homogenate

The testes homogenate of the goat was found to give rise to six precipitation lines in the gel diffusion test with IS 118. Four of the precipitation lines showed strong reaction. Similarly the reaction between testes homogenate of bucks and IS 108 showed six precipitation arcs (Figs. V and VII and Plate 7).

Immunoelectrophoresis of IS 108 and IS 118 with corresponding antigen resulted in production of six precipitation arcs (Fig. VI). Two each of these were found to be located in the region of albumin, beta-globulin and gamma-globulin respectively.

A number of studies have been carried out by various workers on the fertility of different species using antigenic
properties of testes (Isojima et al., 1959; Katsh, 1959) in
guinea-pigs, (Mange and Protzman, 1967; Edwards, 1964 and McLaren,
(1967) studied the antigenic composition of testes extract of mouse.
Their results revealed the presence of eight antigens of which five
were common to epididymis. Gunaga et al. (1970) observed that the
testes of Wistar rats contained three organ specific antigens.

The antigenic properties of testes homogenate was first
demonstrated by Freund et al. (1953). It was found to be an
aspermatogenic antigen which appeared to originate in secondary
spermatocytes. Katsh and Katsh (1961) observed that the
aspermatogenic antigen was distinct from hyaluronidase. Hartree
and Srivastava (1965) extracted and characterized sperm acrosomal
glycoproteins. No relationship was seen between this antigen and
the aspermatogenic factor of testes.

From the work on fertility of goats immunized with
buck semen by Goel et al. (1968) and Pandey (1966), it appears that
testes homogenates possess certain antigens some of which could be
acquired by spermatozoa in ejaculated semen. The testes homogenate
consists of a mixture of populations of spermatogonia, primary
spermatocytes, secondary spermatocytes and spermatids in different
phases of development. Katsh (1959) and Mange (1970) observed that
the spermatogonia, primary spermatocytes and secondary spermatocytes
were antigenic in guinea-pigs, rabbits and cattle.

The existence of aspermatogenic antigen has been
reported in ram testes (Busey, 1966), bull testes (Loos et al., 1968)
and in guinea-pigs (Bishop et al., 1965). The existence of aspermatogenic antigens in the testes of goats has also been investigated separately in the work presented elsewhere in this thesis. These observations supported the existence of several antigenic components in the testes of goats. Some of these might have originated and belonged to the germinal epithelial cell linings. The observations of Katsh (1959) and Menge (1970) support the present findings.

Thus the testes homogenate of buck consists of a mosaic of major and minor antigenic components. These antigens could originate from the various cell types present in male generative organ. Four of these major antigens were highly reactive in-vitro in contrast to the two minor components.

5.6.2. Antigens of epididymal spermatozoa

Precipitation reactions in agar gel with IS 63 and IS 137 revealed the presence of seven precipitation lines (Fig. VII). Five of these appeared as strong distinct lines. Two of these precipitation lines were faint. The testes homogenate of bucks reacted with the IS 63 and showed four precipitation bands.

Rao and Sadri (1960) reported the existence of seven antigens in buffalo epididymal spermatozoa. Three of these antigens were specific to ejaculated spermatozoa. Barker and Amman (1970) proved that epididymal spermatozoa of bulls possessed four antigens. Hunter and Haif (1964) concluded that epididymal spermatozoa of bulls had three antigens that were common to ejaculated sperm.
Menge et al. (1962) detected five antigens on epididymal spermatozoa of rabbits.

Hunter (1969) concluded that two of the sperm coating antigens originated from epididymis. Barker and Amman (1970) also observed that sperm antigens were present in epididymal plasma.

The antigenic profile of epididymal spermatozoa of goats could be due to possession of some of the antigens which originated in the testes. The tissue and secretions of epididymis which are vital for sperm storage and maturation might also be contributing some antigens to the testicular sperm cells. Thus the epididymal spermatozoa could have some of the antigens derived from both testes and epididymis.

The sharing of some of the antigens of testes by epididymal spermatozoa has been supported by a number of workers based on studies of Wistar rats (Gunaga et al., 1970), mouse (Sadri et al., 1967) and bulls (Barker and Amman, 1970). The studies of Hunter and Haff (1964) and Rao and Sadri (1960) agreed with the present findings that four antigens of epididymal sperms were common to those of testes homogenate.

Thus it was concluded that the antigenic properties of epididymal spermatozoa of bucks were due to presence of seven antigenic components, four of which are shared by testes.

5.6.3. Antigens of ejaculated washed spermatozoa

Reaction of immune sera 124 and 132 with homologous washed spermatozoa of bucks resulted in production of four
precipitation lines. Three of these represented the major antigenic components of ejaculated washed spermatozoa of goat. The fourth precipitation line showed the minor component. Immunelectrophoresis of ejaculated washed spermatozoa with immune sera 124 and 132 showed two arcs.

Weil et al. (1956) failed to demonstrate the antigenic specificity of human spermatozoa. The investigation of Rao and Sadri (1959) established that human spermatozoa possessed three sperm specific antigens. The ejaculated spermatozoa were observed to have seven antigenic components. The report of Rao and Sadri (1959) on the sperm specific antigens was in conformity with the occurrence of sperm agglutinins in sera of infertile men (Rumke and Hellinga, 1954; Wilson, 1954).

Hunter (1969) concluded that the antigenicity of ejaculated rabbit spermatozoa was due to presence of two sperm specific antigens of testicular origin. Besides these the spermatozoa possessed sperm coating antigens (SCA) coming from epididymis and other accessory organs of reproduction. Hunter (1969) also suggested that SCA of seminal plasma blocked the reactive sites of sperm antigens. Barker and Amman (1970) identified four sperm specific antigens of bulls.

In most animals, it is not precisely known in which phase of spermatogenesis the male sexual cells acquire antigenic properties (Matousek, 1970). The antigenicity of spermatogenic antigen of guinea-pig testes begins in the male when sexual cells are at the level of secondary spermatocytes (Katsh, 1960). Baum (1959) suggested that antigenic attributes of sperm could begin
with the conversion of nucleo-histones to protamin in sexual cells which were at the end of spermatogenesis.

The results of work reported elsewhere in this thesis on the spermagglutinating and immobilizing antibodies against washed spermatozoa, demonstrated the high antigenicity of ejaculated washed sperms of goats. The immunodiffusion tests showed the existence of four antigenic components. Two of these antigenic components reacted with antiseraum against seminal plasma (Plate 8). The immunoelectrophoretic studies also revealed the presence of two antigens each that reacted separately with antiseraum against ejaculated washed sperm (Fig. VIII) and antiseraum against buck seminal plasma (Plate 9). These antigens of ejaculated washed spermatozoa that reacted with immune sera against seminal plasma could have originated from seminal plasma secretions.

The existence of antigenic components specific to spermatozoa has been established by a number of investigators in different species. Rao and Sadri (1959), Hunter (1969) and Barker and Amman (1970) demonstrated them in human, rabbit and bull spermatozoa respectively.

The present observations on the existence of two sperm specific antigens of goat spermatozoa is parallel to report of Hunter (1969) and Barker and Amman (1970). It therefore appears that two of the four antigenic components of buck spermatozoa were acquired during their passage after being released from testes. The presence of sperm coating antigens in ejaculated spermatozoa has been confirmed by Boettcher (1969, 1971), Weil (1950), Weil and Rodenburg (1962) also suggested that seminal plasma was the source of antigens on spermatozoa.
In the light of these results supported by parallel investigations on antigenic components of ejaculated washed spermatozoa, it appears that the spermatozoa of bucks consist of four antigenic components. At least two of these four might be common to seminal plasma.

5.6.4. Seminal plasma antigens

The seminal plasma of bucks was found to be highly antigenic. The immune sera from rabbits No. 79 and 86 gave rise to eight to ten distinct precipitation lines in gel diffusion. Immuno-electrophoretic analysis showed the presence of eleven components. The different components were located on three regions of immuno-electrophoresis. The component located in the gamma-globulin region showed prominent precipitation lines.

The seminal plasma contains heterogenous mixtures of different components which form a composite secretion of the products of several accessory organs. A number of reports describing the immunogenicity and antigenic characteristics in rabbit semen and seminal plasma have appeared (Weil and Finkler, 1959; Kirton et al., 1966; Menge and Protzman, 1967; Hunter, 1969; Hunter and Norman, 1969). A number of antigens have been shown to be present in rabbit seminal plasma (Menge and Protzman, 1967; Hunter, 1969), some of which behaved as sperm specific.

Agar et al. (1965) detected presence of the ten antigens in seminal plasma of rams. Kulangara (1969) observed six to eleven components in ram seminal plasma.

Rao and Sadri (1959) showed the presence of fifteen different antigens in human seminal plasma. Shulman and Brownson
(1969) concluded that human seminal plasma consisted of twelve different antigenic components.

The results of immunodiffusion and immunoelectrophoresis of buck seminal plasma showed the presence of eight to eleven different components (Fig. IX and Plates 10 to 13). The existence of similar number of antigenic components as seen in the present study has been observed in rams (Agar et al., 1965; and Kulangara, 1969). Parallel studies by various experiments in rabbits confirmed the heterogeneity of seminal plasma secretions. Yantorno (1972) showed that the seminal plasma of rabbits could be resolved into 12 to 14 components using immunoelectrophoresis and polyacrylamide gel electrophoresis.

The variation in the immunoelectrophoretic pattern of seminal plasma has been attributed to storage conditions of samples (Schneider, 1955). Shulman (1969) pointed out that storage conditions and variation in analytical procedures contributed to varying results reported by different workers. The variation in reaction with IS 79 analysed in this study could be due to storage as the samples were kept 8-12 weeks at -15°C. However antiserum 86 gave invariably identical results with fresh samples. The slight variation in minor components observed could be due to variations in the antigenic contents of ejaculates as pointed out by Shulman (1969).

Shulman (1969) and Yantorno et al. (1972) have shown the various components that were located in various mobility regions. The presence of some of the bands appeared similar. At least three bands observed in buck seminal plasma also appeared to show similarity with the location of components in beta-globulin region.
The albumin region was marked by presence of one prominent arc and a few precipitation lines with lighter intensity. In the present work the immunoelectrophoresis showed two prominent arcs (Fig. IX, Plate 13) in the gamma-globulin region. One of these arcs showed bifurcation which appeared to indicate partially similar antigens. These arcs appeared to be more comparable to those shown by Yantorno et al. (1972) in rabbit seminal plasma. One of these could be similar to fraction V. The buck seminal plasma may also contain variable quantities of IgG similar to that of rabbits (Yantorno et al., 1972).

Thus the high antigenicity of buck seminal plasma could be due to presence of a variety of antigens. These eight to eleven antigens detected were found to be located in various mobility regions of immunoelectrophoresis.

5.6.4.1. Immunochemical studies on seminal plasma

Starch gel electrophoresis of buck seminal plasma revealed the presence of six different components (Plate 15, Fig. XI). Studies on fractionation on gel chromatography showed the existence of four peaks (Fig. X). The first peak appeared to possess mobility faster than beta-globulin of goat serum and gave a single arc in immunoelectrophoresis.

The fractionation studies on seminal plasma have been conducted by Yantorno et al. (1972), Shulman (1969) with rabbit and human seminal plasma. These results have shown the presence of six, and five peaks respectively. Shulman made a detailed study and concluded that third fraction was the major peak which possibly
corresponded to sperm coating antigen (SCA) reported by Hekman and Rumke (1969). The first fraction obtained by Yantorno et al. was the major fraction.

In the present study the fraction A (first fraction) was the major component eluted in gel chromatography (Fig. X). Immunoelectrophoretic analysis showed single arc indicating the purity of the component. It was comparable to the fraction I of Yantorno et al. (1972). However further studies are required to elucidate the antigenic nature of peak A of goat seminal plasma.

5.7. Isoimmune antibodies in female goats

High titres of antibodies were observed in sera of does immunized subcutaneously with testes homogenate and ejaculated washed spermatozoa of bucks along with adjuvant. No antibodies were detected in the vaginal mucus of goats immunized intravaginally with testes homogenate and ejaculated washed spermatozoa. The sera of these goats had titres of antibodies varying from 1/16 to 1/128. All the eight animals had normal oestrus and conceived. These does had normal gestation and gave birth to 16 kids, but two of the kids died shortly before or after delivery.

The possibility of controlling fertility by immunization with seminal antigens has been studied by a number of investigators. Infertility in females has been induced by Edwards (1964) and McLaren (1964) in mice. Immunization of females with homologous testes or spermatozoa was reported to cause infertility in guinea-pigs (Itojima et al., 1959; Katsah, 1959b; Behrman et al., 1965). Kiddy et al. (1959) reported the occurrence of fertilization failure and
embryo loss in rabbits inseminated with semen treated with specific immune serum against spermatozoa. Mengs (1967 and 1969) observed that immunization with bull spermatozoa resulted in formation of antibodies that decreased fertility in heifers. Fertility inhibition was caused in rabbits (Mange, 1968; Bell, 1969). Goel et al. (1968) did not observe decrease in the fertility of goats immunized with homologous semen.

Observations by Mange (1967, 1969 and 1970) on lowered fertility in heifers with testes and semen antigens was due to inhibition of fertilization and embryo mortality. Similarly Kiddy et al. (1959) observed that infertility in rabbits inseminated with semen treated with homologous immune serum increased embryo mortality. But from the report of Goel et al. (1968) it appears that hyperimmunization of female goats with semen antigens caused neither fertility inhibition nor embryo mortality.

It seems from the results of Mengs (1967, 1969 and 1970) and Kiddy et al. (1959) that the infertility in rabbits and heifers was due to action of antibodies present in the genital tract. Thus the fertilization inhibition and embryo mortality could result mainly from the reaction of antibodies present at the site of fertilization and implantation. The infertility in rabbits causing embryo mortality (Kiddy et al., 1959) was mainly due to action of antibodies on treated spermatozoa. The insemination of semen treated with antiserum could result in highly diluted suspension of antibodies in the reproductive tract, which may not be enough to cause a direct effect. However Mengs (1969a) observed that fertilization inhibition was due to the action of antibodies both on spermatozoa and ovum.
These results are supported by experiments (Mange, 1969b) that treatment of rabbit blastocyst with isoaosera against spermatozoa results in embryo mortality. Similarly the embryo mortality in females immunized with semen could be due to direct action of antibodies on blastocyst prior to implantation.

However the work of Goel et al. (1968) in goats supported the hypothesis that hyperimmunization did not result in reduction of fertility. The levels of antibodies in the genital tract of the female goats were not measured by these workers. But it looks from their results that the level of antibodies in the reproductive tract might be very low or absent and thereby had no effect either on spermatozoa or the embryo.

The present work agrees with the work of Goel et al. (1968). The absence of antibodies in the vaginal mucus of experimental animals in this work further supports the results of Goel et al. that the normal fertility was due to absence of antibodies in the female genitalia. All the animals that were immunized subcutaneously with testes homogenate had high level of antibodies in the sera. However the antibodies were not detected in the mucus of the hyperimmunized goats. But all the female goats immunized intravaginally with homologous testes homogenate or ejaculated washed spermatozoa had lower level of titres in the serum ranging from 1/16 to 1/128. Only one animal had titre below 1/32 (Table XV). There was no sperm agglutinating activity in the mucus of these goats. Thus in the present investigation, inspite of high level of antibodies in the sera of immunized goats, the antibodies against semen and testes were absent in the genital tract (vagina) secretions. Therefore it seems reasonable to consider that the absence of antibodies in the
genitalia may be due to lack of passage of antibodies from circulation. Kerr (1955) and Kerr *et al.* (1953) observed production of antibodies against *Brucella abortus* and *Trichomonas foetus* in the genital tract of cattle.

From the present work, and that of Goel *et al.* on goats, the hypothesis of local production of antibodies against seminal antigens may not be accepted. The early claims (Hanle and Hanle, 1940; Parsons and Hyde, 1940) on transmission of circulating antibodies against spermatozoa into uterus as a cause of infertility was questioned (Katsh, 1959).

Positive correlation between antibodies in the serum and infertility was observed in mice (McLaren, 1964), rabbits (Behrman and Nakayama, 1965; Rao and Bangalore, 1963) and cows (Mange, 1967; Sokolowskaya and Reshetnikova, 1968). Negative correlation between antibodies in the serum and infertility was observed in mice (Edwards, 1960; McLaren, 1966), and goats (Goel *et al.*, 1968).

Edwards (1969) considered that antibody following immunization could be transmitted and a correlation between serum and genital tract could cause infertility. There are conflicting claims in the literature on the existence of correlation between antibody level in circulation and the female reproductive tract. The variation in the results observed by various workers supporting and denying existence of antibody correlation in serum and mucus could be most probably due to variations in the type of antibodies produced. It was also pointed out that the correct technique to detect the different immunoglobulins has not been applied. Hence there is no direct evidence of reaction between antibody and spermatozoa or seminal plasma in uterus after systemic immunization.
The titres of various antibodies in vaginal and cervical secretions were variable or perhaps nil or sometimes higher than in serum (Edwards, 1969). The source of circulating antibody found after intravaginal immunization is not known.

The results indicated that antibodies against testes homogenate and spermatozoa do not cause fertilization failures in goats. This could be due to lack of transmission of antibodies across the genitalia from circulation, and absence of local production of antibodies against testes and sperm antigens in the genital tract of goats.

5.8. Sperm migration in the female genitalia

Studies on sperm distribution in the genital tract of both the immunized and control groups showed that cervix of goats had highest number of spermatozoa. The total number of sperms in the different segments of uterus was higher than the number in the oviduct. There was one animal (control) which showed slightly higher number in the oviduct than the uterus. The cervical, uterine and oviductal fluids of immunized goats did not cause agglutination of spermatozoa in the genital tract.

Mattner and Braden (1963, 1966 and 1969) observed that sperm migration from cervix to uterus was dependent upon the activity of myometrium. The cervical reserve of sperms served as a reservoir for slower and prolonged phase of transport. Mattner and Braden (1970) observed the effect of time of insemination on distribution
of sperms. The studies on sperm migration and distribution (Blandau, 1969) revealed the importance of physical characteristics and composition of cervical fluids for sperm movement. The studies on sperm migration in the uterus has been investigated by VanDemark and Moellar (1951), Hays and VanDemark (1953), and VanDemark and Hays (1954). Mange et al. (1962), Bedford (1965) and Mattner (1969) studied the phagocytosis of spermatozoa in the genital tract of rabbits and ewes. The capacitation of spermatozoa in the female tract has been studied by Austin (1952). The capacitated sperms undergo acrosomal reaction which released certain enzymes.

The high concentration of spermatozoa in the cervix of both the immunized and control groups (Figs. XII and XIII) observed in this experiment is in agreement with the studies of Mattner (1963, 1966), and Mattner and Braden (1963). It was also observed that the high concentration was as a result of accumulation of inseminated sperms in the cervix of female goats.

The number of sperms obtained from the different segments was much lower than the sperms inseminated. The difference in the number of sperms recorded could be due to either dissolution or phagocytosis of some of the sperms.

It was also observed that a small amount of inseminated semen was expelled by some of the animals from the vulva. This could also cause a great variation in the contents of sperms in the lumen of the segments. The likelihood of a number of sperms sticking to the mucosa of the genital tract and not recovered cannot be ruled out. The differences also could be due to a number of sperms that might
have remained in the vagina and could not be flushed. Thus the
total number of sperms present in the cervix was only an indication
of the sperm population present in both the vagina and cervix. The
same point is also true for different segments of uterus. However,
the number of sperm cells recorded in the oviduct may be a correct
indication of the absolute numbers present in the lumen.

From the studies on sperms distribution in the
different parts of the reproductive tract of the immunized females,
it appeared that there was no significant difference from the
distribution of sperms in the control.

5.9. Experimental immune allergic aspermatogenesis(orchitis)

A dose schedule employing two, four, six and twelve
injections respectively of testes homogenate along with adjuvant
resulted in histological lesions of varying degree in the testes
of experimental bucks as well as physiological and behavioral
changes.

5.9.1. Histological changes in the testes

Immunization of bucks with two and twelve injections
produced more profound changes in cellular lining of seminiferous
tubules of testes than those that were given four and six injections.
There was a decrease in the index weight of testes of immunized
animals especially those receiving twelve injections.

Numerous attempts have been made to produce tissue
specific immune aspermatogenesis. Voisin et al. (1951) and Freund
et al. (1953) succeeded in establishing the main features of the
disease in guinea-pigs. A generalised involvement of the testes was
observed by Freund et al. (1954). The histopathological lesions were graded according to severity. The desquamation of germinal cells and pycnotic formation of multinucleated germ cells were noticed. Freund et al. (1957) proved that the tissue damage was specifically due to immune reaction and not due to action of adjuvant. Bishop (1961) and Katsh (1960) induced the condition in guinea-pigs using auto and isologous tissues. Mancini et al. (1965) and Andrade et al. (1969) produced the disease in man and monkeys respectively. Voisin and Toullot (1968, 1969) demonstrated the complex auto-antigenic system present in guinea-pig spermatozoa.

In the present experiment antibodies were found in the sera of experimental animals which reacted with testes homogenate in gel diffusion tests. Chutna and Rychlikova (1964a) presented evidence of suppression of testicular reaction in guinea-pigs. The damage to the testicular tubules was suppressed either by immunizing the animal before or after the damaging sensitization. The humoral antibody was suggested to counteract or neutralise the cytotoxic antibody causing the lesions. In the studies on bucks with different doses of the antigen, it was particularly observed that bucks No. 120, 130, 20 and 22 that received four and six injections exhibited testicular lesions of minor nature. The severity of reaction in the above cited bucks was much less than in bucks No. 46 and 47 which received only two injections. These variations in the intensity of damage in the seminiferous tubule linings could not be accounted on the number of injections that different experimental subjects received. It appeared that the levels of cytotoxic antibodies present in the animals No. 120, 130, 20 and 22 were probably less than in animals No. 46 and 47.
Protective role of antibodies

Based on the studies of Chutna and Rychlikova (1964a) the occurrence of protective action of humoral antibodies cannot be ruled out. Further Chutna and Rychlikova (1964b) and Bishop and Carlson (1970) have attributed the protective action of cutaneous anaphylactic type of antibodies that are formed in the animal.

From the role of the different kinds of antibodies formed it seems that perhaps the immunological mechanism giving rise to protection comes into play. Thus there is interplay of two opposite forces in the immune disease which affect the testicular tubular linings. The support of this hypothesis is derived from the findings of Matousek (1964) who observed testicular damage of temporary nature which could be reversible in rams. Goat being a phylogenetically related animal, the existence of similar mechanism operating in both the species may be suspected.

Role of immunosuppressive anti-inflammatory agents

It is rational to believe that the protective action of normal sera is due to its content of glucocorticoids from adrenal cortex. To be more precise it seems that delayed hypersensitivity reaction which is due to infiltration of some of the cells of reticuloendothelial system might be inhibited. The pronounced inhibitory effect of thymectomy as observed Pokorna and Vojtikova (1961) and action of immunosuppressive agents which act as anti-inflammatory agents by suppressing the activity of fibroblasts and depression of vascularization resulting in selective permeability which diminishes exudation of plasma (McDonald, 1974). The selective permeability
afforded by the anti-inflammatory cells may also help inhibition of the production of aspermatogenesis by diminishing the binding of antisperm antibody with sperm cells in sperm passage system (Tung et al., 1971) in the testes.

The present studies lend weightage to the hypothesis proposed by Chutna' and Rychlikova' (1964), Bishop and Carlson (1970), Pokorna' and Voj'tiskova' (1964c, 1966). Further it appears that there exists a better protective mechanism in goats as compared to some other laboratory animals.

The degenerative changes in the testes of experimentally immunized goats were similar to those observed by Matousek (1969) in sheep who also noted the absence of infiltrating inflammatory cells. The testicular tissue of goats showed only a few lymphocytes. Therefore the reaction in goats and sheep (Matousek, 1969) appear to be of degeneration. These reactions (in sheep and goats) do not conform to the strict definition of inflammatory reaction. Hence the immune disease in goats may not be orchitis in a strict sense. Therefore the degenerative changes of the testicular tubules of goats may be better designated as immune allergic aspermatogenesis*.

From the results of the study embodied in this thesis on the immune allergic orchitis of Indian goats it seems that this species is equally vulnerable to the immune disease but the protective mechanism to counteract the lesions perhaps is also there. Therefore it is worthwhile to take up further investigations on the role of immunosuppressive and anti-inflammatory agents in affording protection to animals which are highly susceptible and reactive to severe

*The author is grateful to Dr.M.K.Nair, Professor of Pathology at Kerala Agricultural University for pointing out the inappropriateness of the term "Orchitis" in the current context.
5.10. Behavioral studies on bucks (goats)

Experimental investigations on the sexual behaviour of the treated and the control goats were carried out during the three different periods of study. These constituted observations on reaction time and libido of goats prior to immunization, one week after immunization and two weeks after immunization. The majority of experimental animals showed a marked decrease in libido and a noticeable increase in the reaction time. The control animals were unaffected.

Menge (1965) and Yontorno (1972) have studied some of the seminal attributes of the experimentally induced immune aspermatogenic bulls and rabbits. However it seems that there is lack of sufficient information on the sexual behaviour of affected animals. Beach (1970) studied the sexual responsiveness in castrated dogs. The sexual responsiveness was measured by the speed with which a male began to copulate with a receptive female. It was observed that the loss of testicular hormone (castration) had no effect on the copulatory performance of male dogs. The castration did not affect the sexual ability.

Loss of libido and increased reaction time was observed in all the bucks immunized with testes homogenate along with adjuvant. In all these animals the increase in the reaction time was observed to be maximum after two weeks of immunization.

This may be due to the full effect of immunization with different doses of the testes homogenate. A small increase in the
reaction time of some of the animals during the first week of immunization could be due to adaptation mechanism of the animal and time taken by the immune mechanism to exert its full effect and produce its consequence.

LeBoeuff (1967) and Beach and Merari (1968) studied the socio-sexual behaviour of female dogs. Beach (1970) observed that loss of testicular hormones (castration) had no effect on the performance of male dogs. In his experiment the sexual responsiveness of animals was observed for as long as three years. In the present study the lack of libido and increased reaction time were observed during the time when the lesions caused by the immunological reactions were at maximum. The sexual behaviour was expressed in the affected animals but intensity of sexual excitement and responsiveness of the bucks was appreciably lowered. From the study of the reaction time and libido of the affected bucks in the first week after immunization, it appeared that immunization for the first week did not have appreciable effect (Tables XIX to XXII).

There was some sexual response in majority of the animals that were immunized although the animals lacked optimum degree of libido to mount and ejaculate. Further, the damage to the different parts of the testes was not of the same intensity. Even in the animals affected severely, there were some tubules which had somewhat normal histology. Thus the degeneration process varied in severity. These results appeared to agree with the findings of Beach (1970) that the sexual response in dogs did not deteriorate over a long period (up to 3 years).
From the present findings it seems that the altered sexual behaviour in the treated animals was due to immunological reaction in the testes characterized by presence of degenerative changes in the seminiferous tubules. However it may be worthwhile to undertake long time studies spreading to several months to study the sexual behaviour of immune aspermatogenic animals and to investigate cause and effect relationship. The sexual behaviour of affected animals may also serve as one of the criteria for detecting autoagglutinins in infertile subjects.

5.11. Semen characteristics of bucks (goats)

All the semen attributes of experimental bucks except percent dead count did not show an appreciable change during the second and third weeks of the study. A rise in the percent dead count of sperms in the majority of the bucks was seen in second and third weeks of the study. This rise coincided with the period in which the animals sustained testicular lesions and damage.

There is lack of information on the seminal characteristics of experimental animals having immune allergic aspermatogenesis. Yantorno et al. (1972) observed occasional oligospermia and azoospermia in rabbits in which autoimmune orchitis was produced. Semen samples from these rabbits showed no change in sperm number, motility during the period of treatment. Henge (1965) observed a decrease in the number and motility of ejaculated bull spermatozoa in the immunized animals.

The studies on semen characteristics of experimental bucks showed that there was a marked increase in the percentage of
dead spermatozoa after the first week of immunization (Tables XIX to XXII). The trend of increase of percent dead sperms continued for the third phase of experiment in most of the animals. The increase in the dead percent of the spermatozoa of the experimental bucks correlated with lesions in the testes tubules. Although the testicular damage in the animals No. 20, 22, 120 and 130 was not of severe nature, still the percentage of dead sperms showed a rising tendency. This may possibly be due to immune reaction as a result of antibodies. It also reflects the pathological picture of the testes and the reproductive status of the animal as a whole. The present result differs from those of Yantorno et al. (1972) who did not observe a change in viability of rabbit sperms. Menge (1965) did observe a decrease in the number of sperms and the motility of ejaculated semen of bulls. The decrease in the motility of the sperm could be most probably due to mortality of sperms in the ejaculate. Therefore the present finding on the mortality percent of buck spermatozoa in the treated animals agrees with the work of Menge (1965). However there was no decrease in the total count of spermatozoa in the ejaculates of the treated bucks.

Thus the rise in dead percent in the treated bucks could be due to immunological reaction of spermatozoa with antibodies in the genital tract. Therefore the animals in which fewer injections could cause aspermatogenesis, the dead count may serve as an index of the immune disease.

5.12. Immunological response in the epididymis

There was a marked depletion in the contents of spermatozoa in the cut sections of epididymis of the treated animals. Absence of
spermatozoa was seen in the lumen of the tubules.

The response of epididymis has been observed by Vulehanov (1969). Absence of atrophic and degenerative changes noted by previous investigator agreed with the present observations. The normal histology of the tubules of epididymis remained unaffected. The absence of spermatozoa in the lumen of the epididymis of treated animals confirmed the similarity of immune reactions that took place in goats immunized with several dosages of testes homogenate.

Thus the epididymis of the immunized goats did not sustain lesions but a highly decreased number of sperms and the absence of spermatozoa in the experimental animals indicated the pathological changes that affected the testes of goats injected with several dosages of testes homogenate.