Arch. Biol. Med. Exp. 24: 317-329 (1991)
Printed in Chile

Complement biology in the rabbit oviduct and complement inhibition by sulfated oviductal glycoprotein [1]

Biología del complemento en el oviducto del conejo e inhibición del complemento por una proteína sulfatada oviductal.

TED S. THOMAS[2,3], PATRICIA ROSS[2], and RONALD P. TAYLOR[4]

[2] Departments of Obstetrics and Gynecology,
[4] Department of Biochemistry, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA

Others have shown that a majority of rabbit epididymal and ejaculated sperm display naturally-occuring surface antibody without apparent effects on fertility. We have used immunoblotting techniques to demonstrate that complement C4 and C5 are present in native rabbit oviduct fluid (OF) at approximately 10-20% the levels found in serum, suggesting that complement may threaten the viability of antibody-decorated sperm. One mechanism to protect these sperm from complement-mediated damage was proposed by Oliphant *et al.* (*Biol. Reprod. 31:* 205, 1984) in which a specific oviductal secretory component, rabbit sulfated oviductal glycoprotein (SOG) inhibited complement activation *in vitro* and possibly *in vivo*. We have utilized *in vitro* hemolysis and C3b deposition and immune adherence assays to demonstrate that: (1) whole OF and physiological concentrations of purified SOG inhibit the classical pathway of complement activation; (2) one site of SOG inhibition occurs at or prior to the activation of component C3 in the classical pathway; and (3) the alternate pathway of complement activation is unaffected by either whole OF or purifed SOG. Our results support the hypothesis that SOG can function to protect antibody-decorated sperm (and embryos) from classical pathway-mediated complement attack in the oviduct. We suggest that the alternate pathway of complement activation is not inhibited within the oviduct and should, as it does in other body fluids, play a role in the defense against microbial invasion.

INTRODUCTION

Complement is a series of plasma and extra-vascular proteins in higher animals which, when activated, can cause the lysis and/or phagocytosis of targeted cells, local inflammation, directed migration of immune cells, clearance of cellular and molecular complexes from the circulation, and modifications of the immune response (Muller-Eberhard, 1988; Ross, 1986; Frank, 1987; 1988). Complement activation is initiated via one of two different pathways. The classical pathway requires the participation of an antigen: antibody complex, magnesium and calcium. The alternative activation pathway is generally independent of immunoglobulin, requires magnesium, and is initiated by certain carbohydrate structures on bacteria, viruses, and other microbes. In the presence of appropriate stimuli, each pathway proceeds with the sequential proteolytic activation of a distinct set of components. The pathways ultimately converge, however, in the production of activated component C3b followed by the assembly of the membrane attack complex comprised of components C5, C6, C7, C8 and C9. The alternative pathway is considered to play a prominent and vanguard role in the defense mechanisms against infectious disease (Joiner *et al.*, 1984). The classical pathway probably evolved after the alternative pathway and is chiefly associated with antibody-mediated immune phenomena (Frank, 1975).

[1] Funded by Jeffres Foundation Grant J-204 (TST).

[3] To whom correspondence should be addressed

Complement components are most prevalent in serum, but are also present in most extra-vascular fluids including the female reproductive tract fluids of mammals (Oliphant *et al.*, 1977; Price & Boettcher, 1979; Jones *et al.*, 1988; Brown *et al.*, 1990; Schumacher *et al.*, 1965). Reproductive tract complement may have important roles in bacteriostasis and in the clearance of senescent sperm from the reproductive tract lumen (Clark & Klebanoff, 1976), and has been suggested to facilitate the sperm acrosome reaction, although the evidence for the later is both pro (Cabot & Oliphant, 1978; Suárez & Oliphant, 1982) and con (Bedford & Witkin, 1983). The greatest emphasis in the literature is placed on the relationship between reproductive tract complement and the pathological events involved in the autoimmune response to gametes and embryos.

Spontaneous antisperm antibodies are well documented in many species and are often associated with human infertility (Tung, 1977; Bronson *et al.*, 1984; Menge & Naz, 1988). However, antibodies against sperm also exist naturally in serum and in reproductive tract fluids without apparent effects on fertility in the human (Bronson *et al.*, 1982; Tung *et al.*, 1976), mouse (Cohen & Werrett, 1975), guinea pig (Spooner, 1964; Johnson, 1968) and rabbit (Symons & Herbert, 1971; Cohen & Werret, 1975). Some of the natural antibodies are directed against acrosomal or intracellular antigens and are potentially relevant to the removal of moribund sperm from the reproductive tract (Symons, 1967) by mechanisms which could involve complement (Johnson, 1968). Natural antibodies also adhere to the cell surfaces of motile cells, as is the case with ejaculated and epididymal rabbit sperm (Suarez *et al.*, 1981). When exposed to serum complement *in vitro*, rabbit sperm fix complement via a calcium-dependent (classical) pathway and undergo acrosomal loss (Suarez & Oliphant, 1982) or lysis (Hancock, 1976; Edwards, 1960). Rabbit epididymal sperm can also bind complement component C3 in the absence of antibody by the alternate pathway of activation (Witkin *et al.*, 1983). These results suggest that living sperm can be vulnerable to complement attack in the absence of active immunization, and point to the existence of mechanisms to regulate complement activity in both the male and female reproductive tracts.

Complement inhibitors have been reported to exist in rabbit (Suarez & Oliphant, 1982), mouse (Anderson & Tarter, 1982) and human (Tarter & Alexander, 1984; Pedersen *et al.*, 1980) seminal plasma. A complement inhibitor present in human serum (Kirszbaum *et al.*, 1989; Murphy *et al.*, 1989) shares significant homology with specific male reproductive tract secretions in the rat (Griswold *et al.*, 1986; Sylvester *et al.*, 1984), sheep (Rosenoir *et al.*, 1987), and human (Jenne & Tschopp, 1989; Kirszbaum *et al.*, 1989; O'Bryan *et al.*, 1990). At least some of these secretory components adhere to sperm (Sylvester *et al.*, 1984; O'Bryan *et al.*, 1990). Recently, acrosome-reacted human sperm were shown to display a surface molecule related to membrane cofactor protein, a lymphocyte plasma membrane receptor which binds and inactivates complement component C3b (Anderson *et al.*, 1989). These results suggest that both sperm-bound and seminal fluid inhibitors of complement are present when sperm are deposited in the female.

Factors in the female reproductive tract may also regulate complement activation. Oliphant and co-workers demonstrated that rabbit oviduct fluid (OF) collected by intraabdominal flask inhibits both complement-mediated lysis of red blood cells and immobilization of antibody-sensitized rabbit sperm *in vitro*. In addition, antibody-decorated chicken erythrocytes were protected from lysis when surgically deposited in the rabbit oviduct. These assays required specific antibody for lysis, and the oviductal factor or factors were thought to inhibit the classical pathway of complement activation. The inhibitory activity of OF was traced to a specific oviductal secretion referred to as sulfated oviductal glycoprotein or SOG (Oliphant *et al.*, 1984b).

SOG is synthesized under the influence

of estrogen by the non-ciliated epithelial cells of the rabbit oviduct (Erickson-Lawrence *et al.*, 1989a, b). Maximum SOG concentration in OF (1-2 mg/ml) occurs following ovulation and coincides with the presence of sperm and early embryos (Erickson-Lawrence *et al.*, 1989a). Progesterone enhances SOG secretion by oviductal explants *in vitro* (Erickson-Lawrence *et al.*, 1989b). SOG is greater than 200 kDa in native molecular weight (Hanscom & Oliphant, 1976; Oliphant & Ross, 1982), but contains a predominant subunit of 71 kDa following reduction and SDS-PAGE (Erickson-Lawrence *et al.*, 1989a, b).

The current experiments were undertaken to provide further evidence that complement is present in rabbit OF and that the classical pathway of complement activation is inhibited *in vitro* by purified SOG. We further hypothesize that the alternative pathway of complement activation should play a role in maintaining bacteriostasis in the oviductal lumen and should, unlike the classical pathway, be active in the presence of rabbit OF or SOG. Finally, monoclonal antibody against rabbit SOG was used to detect SOG-like molecules in the oviductal secretions of the baboon and human.

MATERIALS AND METHODS

Materials

The following materials were purchased: Sepharose 4B from Pharmacia (Uppsala, Sweden); gelatin, ethylenediamine tetraacetic acid (EDTA), ethyleneglycol-bis-(beta-aminoethyl ether) tetraacetic acid (EGTA), dithiothreitol, hydrogen peroxide, hemolysin, and glycine from Sigma Chemical (St. Louis, MO); barbital and $MgCl_2$ from Fisher Scientific (Fair Lawn, NJ); nitrocellulose sheets from Schleicher and Schuell (Keene, NH); $CaCl_2$ from Mallinckdrodt (New York, NY), acrylamide, bisacrylamide, Tris (hydroxymethyl)-aminomethane from Boehringer Mannheim Biochemicals (Indianapolis, Indiana); diaminobenzidine from Aldrich Chemical (Milwaukee, WI); sodium dodecyl sulfate and ammonium persulfate from Bio-Rad Labs (Richmond, CA); methanol and acetic acid from Scientific Products (Columbia, MD). Peroxidase-conjugated swine anti-goat IgG was obtained from Tago, Inc. (Burlingame, CA) and peroxidase-conjugated goat anti-mouse or anti-rabbit IgG was purchased from Hy Clone (Logan, UT). Antisera against complement components and a non-immune preparation of goat IgG were purchased from The Binding Site (San Diego, CA). The *in vitro* secretions of human (Verhage *et al.*, 1988) and baboon (Verhage & Fazleabas, 1988) oviductal explants, as well as polyclonal antisera against an estradiol-dependent, 130 kDa baboon oviductal secretory glycoprotein (Verhage *et al.*, 1989) were generously provided by Dr. Harold Verhage and co-workers (U. Illinois Medical Center, Department of obstetrics and Gynecology). An Ascites preparation of irrelevant monoclonal IgG antibody was the gift of Dr. John Herr (University of Virginia, Department of Anatomy).

Animals

Sexually mature, estrous New Zealand white rabbits weighing 4 to 5 kg were used for the continuous collection of OF and for the collection of normal rabbit serum complement.

Collection of Rabbit Oviduct Fluid

The intraabdominal flask technique of Hamner and Williams (Hamner & Williams, 1965) was used for the daily collection of OF. The collection flasks were installed under general anesthesia as previously described (Erickson-Lawrence *et al.*, 1989a) and females yielding clear oviduct fluid after 10-14 days of recuperation were included in this study. Approximately 0.7-1.0 ml of OF was obtained daily from each animal. OF was clarified by centrifugation (700 x g, 10 min, 4°C) and used immediately or stored at −20°C.

Isolation of SOG

SOG was purified by affinity chromatography with specific monoclonal anti-

body immobilized on Sepharose according to Erickson-Lawrence *et al.* (1989a). One to two milligrams of SOG was obtained from approximately 10 ml of rabbit OF. Affinity-purified SOG was dialyzed for 24 h against 0.05 M ammonium bicarbonate, pH 7.4, lyophylized overnight, and reconstituted in an appropriate buffer 2-4 prior to use. SOG purified in this manner produces a single predominant band of 71 kDa after reduction, SDS-PAGE, and silver staining.

Serum Preparation

Normal human and rabbit serum complement (NHSC and NRSC, respectively) were prepared from donor blood collected by venipuncture using all plastic syringes (Air-Tite, Inc. Virginia Beach, VA). Serum was clarified from clotted blood by centrifugation at 2000 x g for 15 min and used immediately or stored at −70°C until needed.

Western Immunoblotting

Samples (5-25 μg) of human serum, rabbit serum, rabbit OF, or primate oviductal explant secretions were electrophoresed along with molecular weight standards, electrotransferred to nitrocellulose and processed as described previously (Erickson-Lawrence *et al.*, 1989a; Thomas *et al.*, 1984).

Erythrocyte Collection

Sheep and rabbit erythrocytes (E) were collected by venipuncture into Alsever's buffer (Lennek *et al.*, 1981). Rabbit E were used immediately and sheep E were stored for up to 30 days at 4°C prior to use.

Hemolysis Assay, Classical Pathway

The hemolysis assay was a microadaption of the method of Lennek *et al.* (1981). Equal volumes of a solution of SOG (1, 1.4 or 1.9 mg/ml) and NHSC (dilutions from 1/2 to 1/120) were mixed and incubated for 10 min at 37°C. Subsequently, 20 μl of the mixture was added to 20 μl of a 1.5% suspension of washed antibody-sensitized sheep erythrocytes (EA) (4 μl hemolysin per ml, 30 min 37°C) in gelatin veronal buffer (28 mM sodium barbital, 143 mM NaCl, 0.5% gelatin, pH 7.3, GVB) plus 2 mM $CaCl_2$ and 8 mM $MgCl_2$ in triplicate. After 30 min at 37°C, further complement activity was stopped by the addition of 260 μl of 8 mM EDTA in GVB, the reaction mixture was centrifuged at 8000 x g for 1 min, and the optical density of the supernatant was read at 413 nm. Total and zero hemolysis were determined by incubating triplicates of EA in an appropriate volume of distilled water and in serum dilutions made in GVB-EDTA, respectively.

Hemolysis Assay, Alternate Pathway

The assay for hemolysis of rabbit erythrocytes by the alternate pathway of complement activation was performed following the protocol of Platts-Mills and Ishizaka (1974), except that the volume of the reaction mixture was reduced to 60 μl prior to the addition of EDTA. NHSC dilutions were pre-incubated with and without an equivalent volume of whole OF or with SOG at a final concentration of 0.95 mg/ml.

Complement Fixation, Classical Pathway-dependent Activation of C3

The methodology of Lennek *et al.* (1981) was employed to examine the complement-mediated binding of ^{3}H-DNA: anti-DNA antibody complexes to human E (immune adherence) which requires the sequential activation of components C1, C4, C2, and C3 of the classical pathway. Affinity purified SOG was examined at concentrations of 0.9 and 0.09 mg/ml to determine its ability to inhibit complement activation and immune adherence of DNA/antibody complexes.

C3 Deposition Assay, Alternate Pathway-dependent Activation of C3

A modification of the procedure of Edberg and co-workers (1988) was utilized to test for the alternate pathway-mediated activation of component C3. In this pathway, humoral Factors B, D, H, I and P substitute

for the classical pathway components C1, C4 and C2 (Muller-Eberhard, 1988) in the formation of activated component C3b which is deposited on an appropriate solid phase (Sepharose 4B). Briefly, dilutions (2 to 10-fold) of human serum were incubated in the presence or absence of SOG (final concentration = 0.9 mg/ml) and exposed to Sepharose 4B in Mg-EGTA buffer for 15 min at 37°C. The samples were placed in filter-bottom wells of a multiwell dish and washed extensively by suction filtration. Bound C3b was determined by probing the wells with ^{125}I-labeled Mab to C3b.

Statistical Evaluations

Hemolysis and adherence assay data were plotted as complement activity versus complement dilution. Individual points were the average of triplicate determinations with variability (S.E.M.) noted by error bars. Control and experimental determinations at identical complement dilutions were evaluated by the signed rank test (Colton, 1974). Each assay was repeated 2-5 times and representative results are presented.

RESULTS

Detection of Complement Components in Oviduct Fluid

Antisera directed against human complement components C4 and C5 reacted with bands on immunoblots of rabbit serum and oviduct fluid (Figs. 1 and 2). Anti-human C4 identified the human C4 subunits with molecular mass 93 and 71 kDa and rabbit components at 91 and 71 kDa. Anti-human C5 recognized the human C5 subunits with molecular mass of 115 and 70 kDa, as well as rabbit components at 115 and 91 kDa. Non-immune control sera were unreactive against human and rabbit material. The rabbit serum and oviductal antigens were identical in mobility and similar in intensity when identical amounts of protein were loaded on the gels. Native oviduct fluid collected by intraabdominal flask, however, contains approximately 6-12

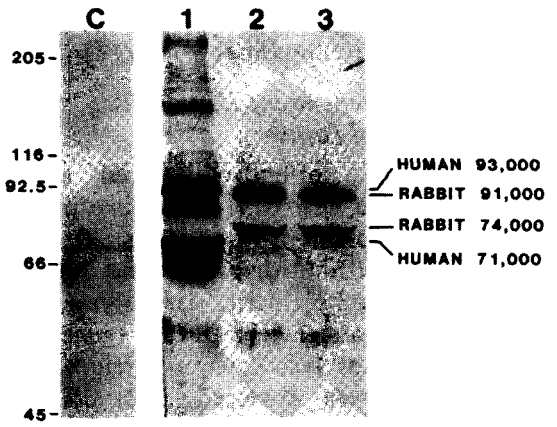

Fig. 1: Presence of complement component C4 in rabbit OF. 2.5 μg of human serum (1), 10 μg of rabbit serum (2), or 10 μg of rabbit oviduct fluid (3) were reduced, subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted with goat anti-human complement C4 or non-immune serum controls (representative in C). Molecular weight standards and the estimated mass of human and rabbit C4 subunits are indicated.

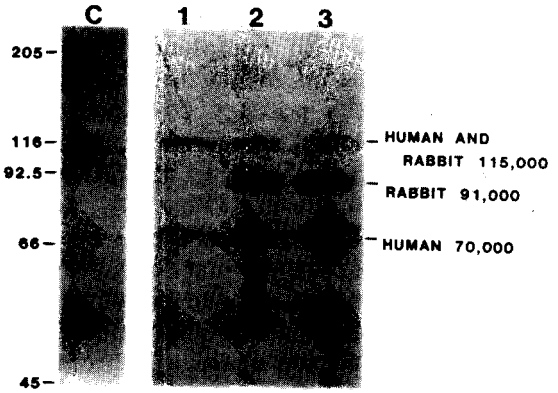

Fig. 2: Presence of complement component C5 in rabbit OF. 2.5 μg of human serum (1), 10 μg of rabbit serum (2), or 10 μg of rabbit oviduct fluid (3) were reduced, subjected to SDS-polyacrylamide gel electrophoresis and immunoblotted using goat anti-human complement C5 or non-immune serum control (representative in C). Molecular weight standards and the estimated mass of human and rabbit C5 subunits are indicated.

mg/ml of protein (Oliphant, 1986) or approximately 1/10 to 1/5 the protein concentration of rabbit serum. Hence, our immunoblots can be crudely interpreted to mean that oviductal complement components C4 and C5 are less abundant than their serum counterparts and are in a range of 10-20% of the serum concentration. These results are in keeping with a report in which rabbit oviductal complement component C3 was determined by radial im-

munodiffusion to be 17% of the serum concentration (Oliphant *et al.*, 1977).

Complement-dependent Hemolysis via the Classical Pathway

The lysis of antibody-sensitized sheep erythrocytes *in vitro* was responsive to the dose of complement used (Fig. 3), and was similar with both rabbit and human sources of complement. Hemolysis was reduced to background levels (generally 10-15% of total lysis) when calcium and magnesium were chelated with EDTA. In the presence of 50% rabbit OF (Fig. 3), complement activity was significantly suppressed ($p < 0.05$) at complement dilutions of 1/33 or greater. The level of SOG in these OF preparations was not determined directly but from previous work (Erickson-Lawrence *et al.*, 1989a), should be in the range of 0.25-0.5 mg/ml after dilution with complement. Affinity purifed SOG was reconstituted in assay buffers and incubated with complement at 0.5, 0.7 and 0.95 mg/ml. While there was some variability in the potency of the various SOG preparations, significant inhibition was noted with 0.95 mg/ml of SOG at all complement dilutions (Fig. 4), lesser inhibition at 0.7 mg/ml (Fig. 5) and no inhibition at 0.5 mg/ml (data not shown). Similar concentration dependency was noted by Oliphant using SOG purifed by gel filtration and ion exchange chromatography in a hemolytic assay with guinea pig complement (Oliphant *et al.*, 1984b).

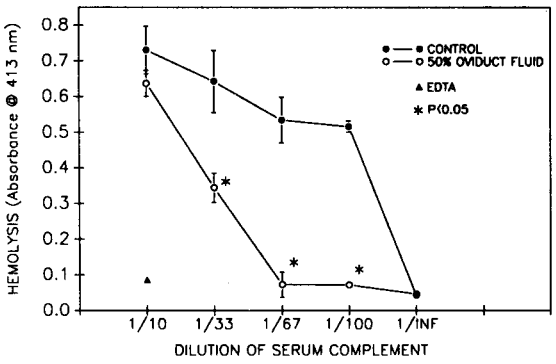

Fig. 3: Complement-dependent hemolysis via classical pathway in the presence or absence of 50% rabbit oviduct fluid. Hemolysis was absent when cations were chelated with EDTA, in the absence of complement (1/INF) or when non-antibody sensitized erythrocytes were exposed to complement (not shown).

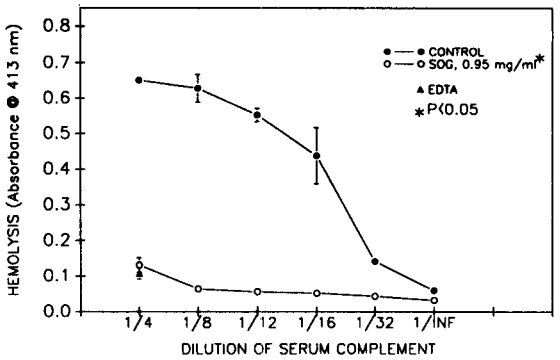

Fig. 4: Complement-dependent hemolysis via classical pathway in the presence of 0.95 mg/ml of affinity purified SOG or control buffer.

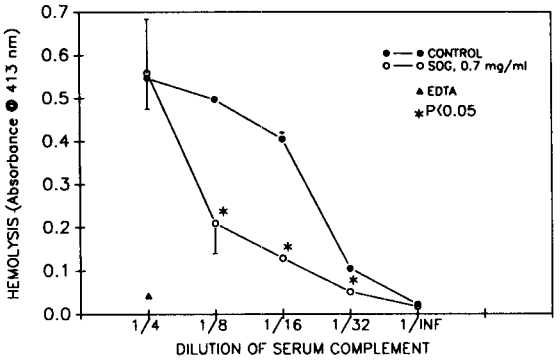

Fig. 5: Complement-dependent hemolysis via classical pathway in the presence of 0.7 mg/ml of affinity purified SOG or control buffer.

Complement-dependent Hemolysis via the Alternate Pathway

The classic assay of Platts-Mills and coworkers (1974) assesses the alternate pathway-dependent hemolysis of rabbit erythrocytes in the presence of Mg-EGTA which precludes the activation of the classical pathway. Dilutions of human complement in MgEGTA gave a dose response of hemolysis as anticipated (Fig. 6). Assays in EDTA, which chelates both calcium and magnesium, abrogated all hemolysis due to inhibition of both the classical and alternate pathways. Affinity purifed SOG (0.95 mg/ml) had no inhibitory effect in this assay (Fig. 6), nor did whole rabbit OF (data not shown).

Classical Pathway-mediated Activation of C3; Complement Fixation

Radiolabeled antigen: antibody complexes were opsonized with NHSC to allow

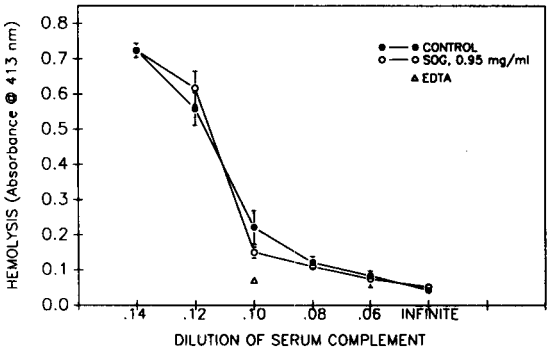

Fig. 6: Complement-dependent hemolysis via the alternate pathway in the presence or absence of 0.95 mg/ml affinity purified SOG. Hemolysis was suppressed in the presence of EDTA, but was unaffected by SOG or by rabbit OF (not shown).

covalent incorporation of C3b and these immune complexes (IC) then bound to erythrocytes via the C3b receptor (Lennek *et al.*, 1981). More than 80% of the IC were cell bound in the presence of NHSC dilutions from 1/2 to 1/50 (Fig. 7). A negative control of radiolabeled antigen and non-immune antibody reduced bound IC to background levels (data not shown), indicating the need for the antigen: antibody complex to activate the classical pathway. In the presence of 0.9 mg/ml of affinity purified SOG, complement-mediated immune adherence was inhibited by 43% and 99% at complement dilutions of 1/10 and 1/50, respectively (Fig. 7). These results indicate that at least one site of SOG inhibition occurs within the classical pathway at or prior to the activation of component C3.

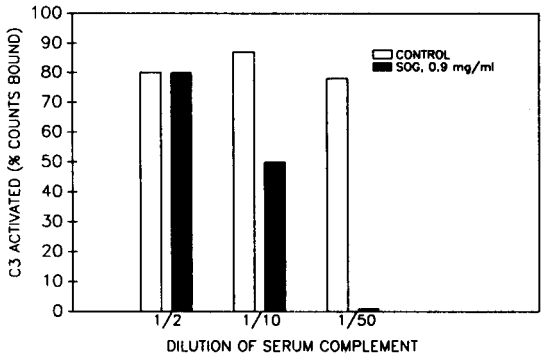

Fig. 7: Classical pathway-mediated activation of C3 was measured by the binding of ^{3}H-labeled antigen: antibody complexes to human erythrocytes in the presence or absence of 0.95 mg/ml of affinity purified SOG. Binding was reduced to background levels (< 10% bound, not shown) when antigen:antibody complexes failed to form in the presence of control non-immune antibody.

Alternate Pathway-mediated Activation and Adherence of C3

The activation of the alternate pathway is stimulated by carbohydrate structures particular to certain microorganisms (Muller-Eberhard, 1988; Joiner *et al.*, 1984). Sepharose 4B effectively mimics these structures and serves as a convenient solid phase for the covalent incorporation of activated C3b (Edberg *et al.*, 1988). The reaction is limited to the alternate pathway in the presence of Mg^{2+} and the absence of Ca^{2+} (chelation with EGTA). Three dilutions of human serum complement resulted in C3b binding to the beads which was unaffected by the presence of 0.9 mg/ml of SOG (Fig. 8).

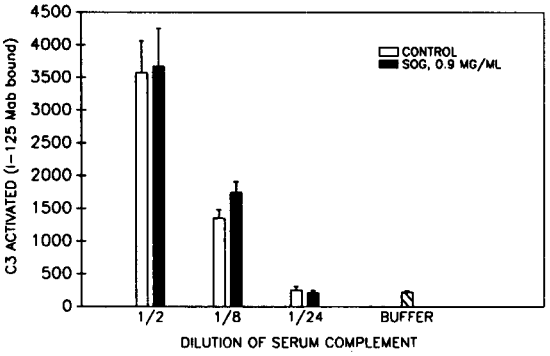

Fig. 8: Alternate pathway-mediated activation of C3 was assessed by the deposition of activated C3b on Sepharose beads in the presence or absence of 0.9 mg/ml of affinity purified SOG. C3b deposition was unaffected by SOG but was suppressed in the presence of EGTA to chelate magnesium (not shown).

Identification of SOG-like Molecules in Primate Oviductal Secretions

Conditioned media from rabbit, human, and baboon oviductal explant cultures were probed on Western blots with Mab specific for rabbit SOG (Erickson-Lawrence *et al.*, 1989a) and with an irrelevant IgG (Fig. 9). Anti-SOG Mab identified a 71 kDa band in both rabbit OF and rabbit oviductal explant culture medium, in agreement with earlier studies (Oliphant & Ross, 1982; Erickson-Lawrence *et al.*, 1989a). In addition, Mab 2 (but not Mab 1 or 3, not shown) identified bands in the oviductal explant culture media of the human at 125 and 145 kDa and of the baboon at 133

kDa. The recognized bands appear to correspond to the estradiol-dependent, oviduct-specific secretory glycoproteins characterized by Verhage and co-workers for both human (Verhage *et al.*, 1988) and baboon (Verhage *et al.*, 1988). Furthermore, antiserum directed against the baboon 130 kDa moiety (Verhage *et al.*, 1989) crossreacts, as expected, with the 71 kDa subunit of SOG in immunoblots of rabbit OF or purified rabbit SOG (data not shown).

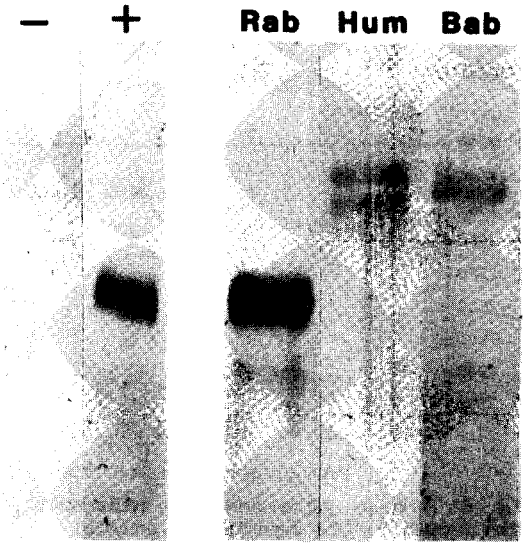

Fig. 9. Immunoblot analysis of anti-rabbit SOG monoclonal antibody against rabbit (rab), human (hum), and baboon (bab) oviductal explant culture medium. Controls included irrelevant antibody (–) and 5 μg of rabbit oviduct fluid collected by intraabdominal flask (+). The estimated Mr of identified bands are: rabbit, 71,000; human, 125,000 and 145,000; and baboon, 133,000.

DISCUSSION

The microenvironment for mammalian fertilization and early embryo development is OF, which is derived from serum transudation and from oviduct-specific secretion (Hamner, 1973). Oviductal secretions are probably important for embryonic development, since embryos develop better *in vitro* when co-cultured with oviductal epithelial cells than with cells of non-oviductal origin (Gandolfi & Moor, 1987; White *et al.*, 1989; Rexroad & Powell, 1988; Whittingham, 1973). Unique components elaborated by the oviduct have been described in the rabbit (Oliphant *et al.*, 1978; Oliphant, 1976), hamster (Robataille *et al.*, 1988; Oikawa *et al.*, 1988), baboon (Verhage & Fazleabas, 1988; Verhage *et al.*, 1989), human (Verhage *et al.*, 1988), pig (Hedrick *et al.*, 1987; Brown & Cheng, 1986), sheep (Sutton *et al.*, 1984), and mouse (Kapur & Johnson, 1985, 1988). Verhage and co-workers have utilized a cDNA to a 130 kDa baboon oviductal glycoprotein to demonstrate the presence of a related mRNA in Northern blots of human, rabbit, hamster, and mouse oviducts (see Chapter by Verhage *et al.*). At the protein level, the glycoproteins encoded by this baboon gene and the apparent human homologue were recognized in the current immunoblotting experiments using Mab specific for rabbit SOG against baboon and human oviduct explant culture media. In addition, antiserum against the baboon 130 kDa oviductal glycoprotein recognizes rabbit SOG on immunoblots. These results suggest that rabbit SOG belongs to a gene family which is conserved in the oviducts of several mammalian species, and that this secretory glycoprotein is of some importance in oviductal physiology.

Mammalian sperm and embryos display antigens to which antibodies are directed in both sexes, in both pathological and non-pathological circumstances. It is the latter instance to which we would like to draw attention. We discussed earlier the existence of naturally-occurring, complement-fixing anti-sperm antibodies in the blood sera and reproductive tract fluids of a number of species showing fertility. Spontaneous antisperm antibodies are also present on the sperm cell surface of rabbit sperm in native reproductive tract fluids, also without apparent effects on fertility. One can ask why antibody-coated sperm are not attacked by complement? Three hypotheses may be offered and include: (I) the antibody associated with the sperm is not able to activate complement; (II) the antibody-sensitized sperm do not

encounter complement *in vivo*; or (III) there is complement within the reproductive tract milieu but that it is inhibited.

Regarding the first hypothesis, the natural antibody bound to rabbit sperm appears to include the IgG and/or IgM classes due to Protein-A recognition (Suarez *et al.*, 1981), and the bound IgG was estimated by radioimmune assay at 10^6 molecules per spermatozoon (Suarez & Oliphant, 1982). This level of antibody is sufficient to stimulate complement-mediated lysis of erythrocytes (Humphrey & Dourmashkin, 1965). Sperm from normal rabbits can fix complement by the classical pathway (Suarez & Oliphant, 1982) and are immobilized when exposed to native, but not heat inactivated homologous serum complement (Hancock, 1976; Edwards, 1960; Chang, 1947), thus suggesting that these natural antibodies pose a significant risk for sperm when exposed to active complement. Alternatively, the natural immunoglobulin coating of rabbit or other mammalian sperm could serve as protective or blocking antibodies to shield privileged cells such as sperm from immune attack (Tung, 1977).

Complement is present in maximal concentrations in the blood, but is also found in almost every extravascular fluid in some amount as a result of transudation and/or local production (Frank, 1975, 1987). Despite this, there is little evidence to suggest that complement is present within the male reproductive tract lumenal environment, except perhaps in the rete testis (Johnson & Setchell, 1968). It is possible that tight junctional complexes restrict the transepithelial migration of complement into the testicular and epididymal lumina. In contrast, sperm do encounter complement within the female reproductive tract. Lytic complement activity has been reported in the cervical mucus environment of humans (Price & Boettcher, 1979). In the uterine environment, bioactive complement component C5 has been identified in the rabbit (Jones *et al.*, 1988), and the rat and human uterine epithelia have been shown to secrete component C3 (Brown *et al.*, 1990; Isaacson *et al.*, 1990). In rabbit OF, complement component C3 was previously identified using double diffusion (Oliphant *et al.*, 1977) and in the present work, components C4 and C5 were also identified by immunoblotting of whole OF. Attempts to demonstrate complete complement bioactivity (hemolytic activity) in rabbit OF collected by the flask method demonstrated nonexistent or very low levels of activity (Oliphant *et al.*, 1977; Cabot & Oliphant, 1978). This is not surprising given the endogenous complement inhibiting activity of OF (see below). Taken together, these results suggest that complement is present within the female reproductive tract milieu and that, in the absence of complement inhibiting factors, antibody-coated sperm or embryos would be vulnerable to complement-mediated attack.

The third hypothesis suggests that one method of protecting sperm from the deleterious effects of surface antibody is to inhibit the complement-mediated lysis of antibody-decorated cells. We reviewed earlier the evidence for distinct inhibitors of complement activation originating in male reproductive tract fluids and on spermatozoa and it is possible that these factors serve to regulate complement in the female reproductive tract. Oliphant and co-workers were the first to show that maternal factors could also inhibit complement activation (guinea pig) *in vitro* and that this may contribute to the immune privileged condition of sperm and embryos (Oliphant *et al.*, 1984b; Oliphant, 1986). Using an antibody-dependent hemolysis assay, the present study has demonstrated that OF and SOG inhibit rabbit and human serum complement, as would be expected due to the highly conserved nature of the complement system in higher animals. SOG inhibition of the classical activation pathway was observed at a concentration of 0.95 mg/ml, which is in the middle of the range of SOG concentrations observed in whole oviduct fluid following ovulation (Erickson-Lawrence *et al.*, 1989a). Complement inhibition was reduced at lesser concentrations of SOG and was absent at 0.5 mg/ml.

Fifty percent whole OF collected from estrous females, with endogenous SOG of approximately 0.25-0.5 mg/ml, displayed significant inhibition in the hemolysis assay, suggesting that additional complement inhibitors are present in OF or that some loss of inhibitory activity is associated with the isolation and reconstitution of affinity purified SOG. We favor the latter explanation since complement inhibiting activity was rapidly lost from affinity purified SOG following certain denaturing conditions such as bubbling, freezing, or extended refrigeration (data not shown).

Purified SOG inhibited classical pathway-mediated hemolysis at serum complement dilutions as high as 1/4. The level of complement activity is not known in the rabbit oviduct but is undoubtedly less than the level in serum. Complement component C3 was determined in rabbit OF by immunodiffusion to be approximately 1/5 of the serum level (Oliphant *et al.*, 1977), and serum macromolecules in general were found in OF at 5-10% of serum concentrations (Iritani, 1969). In the present immunoblotting experiments, crude estimates of the levels of components C4 and C5 in rabbit oviduct fluid also fell in this range. Hence, the concentrations of serum complement which were inhibited *in vitro* are in a range which is physiologically relevant to the levels which are likely present in OF.

In contrast to the inhibitory effects of SOG on the classical pathway, neither SOG nor OF displayed inhibition of the alternative pathway in an *in vitro* hemolysis assay. This suggested that the site of SOG inhibition was not among the activation steps which are shared by the two pathways (C5 through C9). The activation of C5 is through the activity of a C5 convertase. In the alternate pathway, Factors B, D, H, I and P and component C3 initiate and amplify the events which lead to the formation of the C5 convertase (Muller-Eberhard, 1988). In the classical pathway, the convertase is derived from the activation of components C1, C4 and C3, (Hourcade *et al.*, 1989) and it is this sequence which we suggest to be affected by SOG. In order to test this hypothesis, we assessed the activation of the first four components of the classical pathway in an immune adherence assay (requiring activated C3b) in the presence or absence of SOG. As predicted, 0.9 mg/ml of SOG inhibited the complement-mediated adherence of antigen: antibody complexes to erythrocytes, strengthening the hypothesis that the site of SOG-mediated inhibition involves one or more of the first four activation steps of the classical pathway. Also as predicted, SOG did not effect the early steps of the alternate pathway assayed by the covalent incorporation of C3b into Sephadex beads.

There are numerous inhibitors of the early steps of complement activation which either prevent the activation or accelerate the degradation of specific complement components (Reid, 1986; Muller-Eberhard, 1988; Hourcade *et al.*, 1989). In the classical pathway, one family of inhibitors is focused on the steps which activate components C4 or C3 and have in common a 60 amino acid repeat in primary structure (Muller-Eberhard, 1988; Hourcade *et al.*, 1989). This family includes Factor H, C4-binding protein, decay accelerating factor, membrane cofactor protein, and the C3 receptors present on some blood cells (CR1 and CR2). Several inhibitors of component C1 have also been identified and characterized (Muller-Eberhard, 1988; Lopez-Trascasa *et al.*, 1989). It is not known whether SOG is related to one of the known complement inhibitors or is a unique regulator of complement activation. Efforts to elucidate the structure of SOG and the biochemical mechanisms involved in SOG-mediated complement inhibition are ongoing in our laboratory.

The alternative pathway of complement activation is thought to function as a first line of defense against microbial invasion in essentially all vascular and extravascular fluids (Frank, 1987; Joiner *et al.*, 1984). We hypothesize that this would likely also be the case in the female reproductive tract, where infection can lead to scarring, occlusion and subfertility or sterility (Svensson *et al.*, 1983). This seems especially likely in view of the bacterial and viral

components which accompany the mammalian ejaculate upon deposition in either the vagina or, in some species, the uterus. These microbial elements could invade the oviduct by diffusion, by adherence to ascending sperm, or by the mechanisms which transport sperm to the site of fertilization. An element of proof that the alternate pathway of complement plays a role in reproductive tract bacteriostasis would be to demonstrate the functional activity of this pathway in native reproductive tract fluids. Our attempts to do this with rabbit OF have been thwarted by the lengthy period (24 hours) during which the needed volume of OF collects in the flask. In this time frame, any complement activity present would likely autoactivate or degrade to very low levels due to the labile nature of some components such as C3 (Harrison & Lachman, 1980). In an alternative approach, we have shown that neither rabbit OF nor purified SOG are inhibitors of alternate pathway activity *in vitro*, using hemolytic and C3b deposition assays. Hence, our findings support the hypothesis that the OF environment inhibits the classical pathway of complement activation but does not inhibit the alternative pathway.

REFERENCES

ANDERSON, D.J.; MICHAELSON, J.S.; JOHNSON, P.M. (1989) Trophoblast/Leukocyte-common antigen is expressed by human testicular germ cells and appears on the surface of acrosome-reacted sperm. *Biol. Reprod. 41:* 285.

ANDERSON, D.J.; TARTER, T.H. (1982) Immunosuppressive effect of mouse seminal plasma components *in vivo* and *in vitro*. *J. Immunol. 128:* 535.

BEDFORD, J.M.; WITKIN, S.S. (1983) Influence of complement depletion on sperm function in the female rabbit. *J. Reprod. Fert. 69:* 523.

BRONSON, R.A.; COOPER, G.W.; ROSENFELD, D.L. (1982) Correlation between regional specificity of antisperm antibodies to the spermatozoan surface and complement-mediated sperm immobilization. *Am. J. Reprod. Immunol. 2:* 222.

BRONSON, R.; COOPER, G.; ROSENFELD, D. (1984) Sperm antibodies: their role in infertility. *Fert. Steril. 42:* 171.

BROWN, C.R.; CHENG, W.K.T. (1986) Changes in composition of the porcine zona pellucida during development of the oocyte to the 2-to 4-cell stage. *J. Exp. Morphol. 92:* 183.

BROWN, E.O.; SUNDSTROM, S.A.; KOMM, B.S.; YI, Z.; TEUSCHER, C.; LYTTLE, R.C. (1990) Progesterone regulation of estradiol-induced rat uterine secretory protein, complement C3. *Biol. Reprod. 42:* 713.

CABOT, C.L.; OLIPHANT, G. (1978) The possible role of immunological complement in induction of rabbit sperm acrosome reaction. *Biol. Reprod. 19:* 666.

CHANG, M.C. (1947) The effects of serum on spermatozoa. *J. Gen. Physiol. 30:* 321.

CLARK, R.A.; KLEBANOFF, S.J. (1976). Generation of a neutrophil chemotactic agent by spermatozoa: Role of complement and regulation by seminal plasma factors. *J. Immunol. 117:* 1378.

COHEN, J.; WERRETT, D.J. (1975) Antibodies and sperm survival in the female tract of the mouse and rabbit. *J. Reprod. Fert. 42:* 301.

COLTON, T. (1974). *Statistics in Medicine.* Chapter 7. Little Brown Co., Boston, M.A. pp. 219.

EDBERG, J.C.; TOSK, L.; WRIGHT, E.L.; SUTHERLAND, W.M.; TAYLOR, R.P. (1988) Quantitative analyses of the relationship between C3 consumption, C3b capture, and immune adherence of complement-fixing antibody/DNA immune complexes. *J. Immunol. 141:* 4258.

EDWARDS, R.G. (1960) Complement-fixing activity of normal rabbit serum with rabbit spermatozoa and seminal plasma. *J. Reprod. Fert. 1:* 268.

ERICKSON-LAWRENCE, M.F.; TURNER, T.T.; ROSS, P.R.; THOMAS, T.S.; OLIPHANT, G. (1989a) Sulfated oviductal glycoproteins in the rabbit: quantitation by competitive enzyme-linked immunosorbent assay. *Biol. Reprod. 40:* 1299-1310.

ERICKSON-LAWRENCE, M.F.; TURNER, T.T.; THOMAS, T.S.; OLIPHANT, G. (1989b) Effect of steroid hormones on sulfated oviductal glycoprotein secretion by oviductal explants *in vitro*. *Biol. Reprod. 40:* 1311-1319.

FRANK, M.M. (1975) *Complement.* Scope Publications/ Upjohn Co.

FRANK, M.M. (1987) Complement in the Pathophysiology of human disease. *New. Eng. J. Med. 316:* 1525.

FRANK, M.M. (1988) The complement system. In: *Immunological Diseases.* Part One. *Basic Immunology.* Talmage, D.W. ed. Little Brown, Boston, pp. 203.

GANDOLFI, F.; MOOR, R.M. (1987) Stimulation of early embryonic development in the sheep by coculture with oviduct epithelial cells. *J. Reprod. Fert. 81:* 23.

GRISWOLD, M.D.; ROBERTS, K.P.; BISHOP, P.D. (1986) Purification and characterization of a sulfated glycoprotein secreted by Sertoli cells. *Biochemistry 25:* 7265.

HAMNER, C.E. (1973) Oviductal fluid: Biochemistry and Physiology. In: *Handbook of Physiology. Endocrinology.* Sect. II, Part. II. Greep, R.O.; Astwood, E.B. (eds.) Washington, D.C. *Amer. Physiol. Soc.* p. 141.

HAMNER, C.E.; WILLIAMS, W.L. (1965) Composition of rabbit oviductal secretions. *Fert. Steril. 16:* 170.

HANCOCK, R.J.T. (1976) New observations on the spermotoxic properties of normal rabbit serum. *J. Reproduc. Fert. 46:* 435.

HANSCOM, D.R.; OLIPHANT, G. (1976) Hormonal regulation of incorporation of ^{35}S into macromolecules of oviduct fluid. *Biol. Reprod. 14:* 599.

HARRISON, R.A.; LACHMAN, P.J. (1980) The physiological breakdown of the third component of human complement. *Molec. Immunol. 17:* 9.

HEDRICK, J.L.; WARDRIP, N.T.; BERGER, T. (1987) Differences in the macromolecular composition of the zona pellucida isolated from pig oocytes, eggs, and zygotes. *J. Exp. Zool. 241:* 259.

HOURCADE, D.; HOLERS, V.M.; ATKINSON, J.P. (1989) The regulators of complement activation (RCA) gene cluster. *Adv. Immunol. 45:* 381.

HUMPHREY, J.H.; DOURMASHKIN, R.R. (1965) Electron microscope studies of immune cell lysis. In: *CIBA Foundation Symposium on Complement.* Little Brown Co, Boston. p. 175.

IRITANI, A.; NISHIKAWA, Y.; GOMES, W.R.; VAN DEMARK, N.L. (1969) Secretion rates and chemical composition of oviduct and uterine fluids in rabbits. *J. Anim. Sci. 33:* 829.

ISAACSON, K.B.; GALMAN, M.; COUTIFARIS, C.; LYTTLE, C.R. (1990) Endometrial synthesis and secretion of complement component-3 by patients with and without endometriosis. *Fert. Steril. 53:* 836.

JENNE, D.E.; TSCHOPP, J. (1989) Molecular structure and functional characterization of a human complement cytolysis inhibitor found in blood and seminal plasma: Identity to sulfated glycoprotein 2, a constituent of rat testis fluid. *Proc. Natl. Acad. Sci. USA 86:* 7123.

JOHNSON, M.H. (1968) Characterization of a natural antibody in normal guinea pig serum reacting with homologous spermatozoa. *J. Reprod. Fert. 16:* 503.

JOHNSON, M.H.; SETCHEL, B.P. (1968) Protein and immunoglobulin content of rete testis fluid of rams. *J. Reprod. Fert. 17:* 403.

JOINER, K.A.; BROWN, E.J.; FRANK, M.M. (1984) Complement and bacteria: Chemistry and biology in host defense. *Annu. Rev. Immunol. 2:* 461.

JONES, M.A.; KOLB, W.P.; HARPER, M.J.K. (1988) The presence of the fifth component of complement (C5) in rabbit uterine flushings in relation to reproductive state. *Immunol. Invest. 17:* 63.

KAPUR, R.P.; JOHNSON, L.V. (1985). An oviductal fluid glycoprotein associated with ovulated mouse ova and early embryos. *Develop. Biol. 112:* 89.

KAPUR, R.P.; JOHNSON, L.V. (1988) Ultrastructural evidence that specialized regions of the murine oviduct contribute a glycoprotein to the extracellular matrix of mouse oocytes. *Anat. Rec. 221:* 720.

KIRSZBAUM, L.; SHARPE, J.A.; MURPHY, B,; D'APICE, J.F.; CLASSON, B.; HUDSON, P.; WALKER, I.D. (1989) Molecular cloning and characterization of the novel, human complement-associated protein, SP-40, 40: a link between complement and reproductive systems. *EMBO. J. 8:* 711.

LENNEK, R.; BALDWIN, A.S. JR.; WALLER, S.J.; MORLEY, K.W.; TAYLOR, R.P. (1981) Studies of the physical biochemistry and complement-fixing properties of DNA/anti-DNA immune complexes. *J. Immunol. 127:* 602.

LOPEZ-TRASCASA, M.; BING, D.H.; RIVARD, M.; NICHOLSON-WELLER, A. (1989) Factor J: Isolation and characterization of a new polypeptide inhibitor of complement C1. *J. Biol. Chem. 264:* 16214.

MENGE, A.C.; NAZ, RK (1988). Immunologic reactions involving sperm cells and preimplantation embryos. *A. J. Reprod. Immunol. 18:* 17.

MULLER-EBERHARD, H.J. (1988) Molecular organization and function of the complement system. *Annu. Rev. Biochem. 57:* 321.

MURPHY, B.F.; SAUNDERS, J.R.; O'Bryan, M.K.; KIRSZBAUM, L.; WALDER, I.D.; D'APICE, A.J.F. (1989) SP-40, 40 is an inhibitor of C5b-6 initiated hemolysis. *Int. Immunol. 1:* 551.

O'BRYAN, M.K.; BAKER, H.W.G.; SAUNDERS, J.R.; KIRSZBAUM, L.; WALKER, I.D.; HUDSON, P.; LIU, D.Y.; GLEW, M.D.; D'APICE, A.J.F.; MURPHY, B.F. (1990) Human seminal clusterin (SP-40, 49): Isolation and characterization. *J. Clin. Invest. 85:* 1477.

OIKAWA, T.; SENDAI, Y.; SHO-ICHINO, K.; YANAGIMACHI, R. (1988) A glycoprotein of oviductal origin alters the biochemical properties of the zona pellucida of hamster egg. *Gam. Res. 19:* 113.

OLIPHANT, G. (1986) Biochemistry and immunology of oviductal fluid. In: *The Fallopian Tube: Basic Studies and Clinical Contributions.* Siegler, A.M., edit. Futura Pub. p. 129.

OLIPHANT, G.; BOWLING, A.; ENG, L.E.; KEEN, S.; RANDALL, P.A. (1978) The permeability of rabbit oviduct to proteins present in the serum. *Biol. Reprod. 18:* 516.

OLIPHANT, G.; CABOT, C.; ROSS, P.; MARTA, J. (1984a) Control of the humoral immune system within the rabbit oviduct. *Biol. Reprod. 31:* 205.

OLIPHANT, G.; RANDALL, P.; CABOT, C.L. (1977) Immunological components of rabbit Fallopian tube fluid. *Biol. Reprod. 16:* 463.

OLIPHANT, G.; REYNOLDS, A.B.; SMITH, P.F.; ROSS, P.R.; MARTA, J.S. (1984b) Immunocytochemical localization and determination of hormone-induced synthesis of the sulfated oviductal glycoproteins. *Biol. Reprod. 31:* 165.

OLIPHANT, G.; ROSS, P.R. (1982) Demonstration of production and isolation of three sulfated glycoproteins from the rabbit oviduct. *Biol. Reprod. 26:* 537.

PEDERSEN, B.H.; LAMMEL, C.J.; STITES, D.P.; BROOKS, G.F. (1980) Human seminal plasma inhibition of complement. *J. Lab. Clin. Med. 96:* 582.

PLATTS-MILLS, T.A.E.; ISHIZAKA, K. (1974) Activation of the alternate pathway of human complement by rabbit cells. *J. Immunol. 113:* 348.

PRICE, R.J.; BOETTCHER, B. (1979) The presence of complement in human cervical mucus and its possible relevance to infertility in women with complement-dependent sperm-immobilizing antibodies. *Fert. Steril. 32:* 61.

REID, K. (1986) Activation and control of the complement system. *Essays Biochem. 22:* 27.

REXROAD, C.E., JR.; POWELL, A.M. (1988) Co-culture of ovine ova with oviductal cells in medium 199. *J. Anim. Sci. 66:* 947.

ROBITAILLE, G.; ST. JACQUES, S.; POTIER, M.; BLEAU, G. (1988) Characterization of an oviductal glycoprotein associated with the ovulated hamster oocyte. *Biol. Reprod. 38:* 687.

ROSENOIR, J.; TUNG, P.S.; FRITZ, I.B. (1987) Biosynthesis and secretion of clusterin by ram testis cell-enriched preparations in culture. *Biol. Reprod. 36:* 1313.

ROSS, G. (1986) *Immunobiology of the Complement System.* Academic Press, New York.

SCHUMACHER, G.F.B.; STRAUSS, E.K.; WIED, G.L. (1965) Serum proteins in cervical mucus. *Am. J. Obstet. Gynecol. 91:* 1035.

SPOONER, R.L. (1964) Cytolytic activity of the serum of normal male guinea pigs against their own testicular cells. *Nature 202:* 915.

SUAREZ, S.S.; OLIPHANT, G. (1982) Interaction of rabbit spermatozoa and serum complement components. *Biol. Reprod. 27:* 473.

SUAREZ, S.S.; HINTON, B.T.; OLIPHANT, G. (1981) Binding of a marker for immunoglobulin to the surface of rabbit testicular, epididymal, and ejaculated spermatozoa. *Biol. Reprod. 25:* 1091.

SUTTON, R.; NANCARROW, C.D.; WALLACE, A.L.C.; RIGBY, N.W. (1984) Identification of an oestrous-

associated glycoprotein in oviductal fluid of the sheep. *J. Reprod. Fert. 72:* 415.

SVENSSON, L.; MARDH, P.A.; WESTROM, L. (1983) Infertility after acute salpingitis with special reference to Chlamydia trachomatis. *Fertil. Steril. 40:* 322.

SYLVESTER, S.R.; SKINNER, M.K.; GRISWOLD, M.D. (1984) A sulfated glycoprotein synthesized by Sertoli cells and by epididymal cells is a component of the sperm membrane. *Biol. Reprod. 31:* 1087.

SYMONS, D.B.A. (1967) Reaction of spermatozoa with uterine and serum globulin determined by immunofluorescence. *J. Reprod. Fert. 14:* 163.

SYMONS, D.B.A.; HERBERT, J. (1971) Incidence of immunoglobulins in fluids of the rabbit genital tracts and the distribution of IgG-globulin in the tissues of the female tract. *J. Reprod. Fert. 24:* 55.

TARTER, T.H.; ALEXANDER, N.J. (1984) Complement-inhibiting activity of seminal plasma. *Am. J. Reprod. Immunol. 6:* 28.

THOMAS, T.S.; REYNOLDS, A.B.; OLIPHANT, G. (1984) Evaluation of the site of synthesis of rabbit sperm acrosome stabilizing factor using immunocytochemical and metabolic labeling techniques. *Biol. Reprod. 30:* 693.

TUNG, T.S.K. (1977) The nature of antigens and pathogenetic mechanisms in autoimmunity to sperm. *Clin. Exp. Immunoreprod. 4:* 157.

TUNG, T.S.K.; COOKE, W.D.; McCARTY, T.A.; ROBITAILLE, P. (1976) Human sperm antigens and antisperm antibodies. II. Age-related incidence of antisperm antibodies. *Clin. Exp. Immunol. 25:* 73.

VERHAGE, H.G.; FAZLEABAS, A.T. (1988) The *in vitro* synthesis of estrogen-dependent proteins by the baboon (*Papio anubis*) oviduct. *Endocrinology 123:* 552.

VERHAGE, H.G.; FAZLEABAS, A.T.; DONNELLY, K. (1988) The *in vitro* synthesis and release of proteins by the human oviduct. *Endocrinology* 122: 1639.

VERHAGE, H.G.; BOICE, M.L.; MAVROGIANIS, P.; DONNELLY, K.; FAZLEABAS, A.T. (1989) Immunological characterization and immunocytochemical localization of oviduct specific glycoproteins in the baboon (*Papio anubis*). *Endocrinology 124:* 2464.

WHITE, K.L.; HEHNKE, K.; RICHARDS, L.F.; SOUTHERN, L.L.; THOMPSON, JR. D.L.; WOOD, T.C. (1989 Early embryonic development *in vitro* by coculture with oviductal epithelial cells in pigs. *Biol. Reprod. 41:* 425.

WHITTINGHAM, D.G. (1973) Development of zygotes in cultured mouse oviducts. I. The effect of varying oviductal conditions. *J. Exp. Zool. 169:* 391.

WITKIN, S.S.; RICHARDS, J.M.; BEDFORD, J.M. (1983) Influence of epididymal maturation on the capacity of hamster and rabbit spermatozoa for complement activation. *J. Reprod. Fert. 69:* 517.