Lateral Regionalization and Diffusion of a Maturation-dependent Antigen in the Ram Sperm Plasma Membrane

David E. Wolf,* Stephanie S. Hagopian,* Richard G. Lewis,* Josef K. Voglmayr,* and Grant Fairbanks

*Endocrine Reproductive and *Cell Biology Groups, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545. Dr. Voglmayr's present address is Division of Reproductive Biology, Florida Institute of Technology, Melbourne, Florida 32901.

Abstract. We have used a monoclonal antibody ESA152 in fluorescence recovery after photobleaching (FPR) studies of a maturation-dependent surface antigen of ram sperm. The antibody is an immunoglobulin G secreted by a hybridoma derived from NS1 mouse myeloma cells. The ESA152 antigen is not detectable in testicular sperm. It is localized on the surface of ejaculated sperm where it is present on all regions of the surface, but tends to be concentrated on the posterior region of the head. The ESA152 antigen can be extracted by detergents or chloroform-methanol. The extracted antigen is sensitive to proteases and migrates with an apparent Mr ~ 30,000 in SDS-containing 10–20% polyacrylamide gradient gels. FPR measurements of ESA152 lateral mobility in the membrane yield diffusion coefficients in the range $10^{-9}$–$10^{-8}$ cm$^2$/s, values typical of lipids but observed for proteins only at the fluid dynamic limit where diffusion is controlled by lipid fluidity. Immobile fractions, typical of membrane proteins, are observed on all regions. When the antigen is stained by a fluoresceinated Fab fragment of the ESA152 antibody, the diffusibility is highly regionalized, with particularly low, but rapid, recovery on the midpiece. Cross-linking of the antigen with the intact ESA152 antibody induces a redistribution in which the antigen is excluded from the posterior head region. This cross-linking is accompanied by increases in ESA152 diffusibility on both the anterior head and the midpiece.

Since the experiments of Frye and Edidin (16), which demonstrated the ability of surface antigens to intermix upon heterokaryon fusion, it has been clear that cell plasma membrane components are generally free to diffuse within the plane of the membrane. The development of fluorescence recovery after photobleaching (FPR) has enabled us to quantitate diffusion of membrane proteins and lipids in a number of cell systems (for reviews see references 8, 11, and 35). Diffusion is a random process (12). Yet, during processes of cellular differentiation, such as sperm maturation and capacitation (4), preimplantation embryogenesis (22, 52), erythropoiesis (37), epithelial tight junction formation (29, 43), and myotube development (2), cells overcome the randomizing effect of diffusion and regionalize the distribution of certain membrane components. The ubiquity of surface regionalizations during cellular differentiation suggests a close relationship between these two phenomena, and demonstrates the need to understand how cells restrain the free diffusion of their surface components if one hopes to understand the processes of differentiation.

Some of the most dramatic examples of surface component regionalization and its relationship to cellular differentiation are exhibited by mammalian sperm. Sperm localize a number of surface components: antigens (13, 20, 23, 25, 30, 38, 39, 45, 46), enzymes (for review see reference 23), lectin receptors (23, 30, 32, 33), charged lipids (4, 15), and cholesterol (4, 15) to specific surface regions. Some of these patterns of regionalization are transformed during the differentiative processes of epididymal maturation (19, 32) in the male tract and capacitation (4, 34) in the female tract. These processes result in the functional differentiations that lead to the acquisition of motility, the acrosome reaction, and fertilization competence.

Recent FPR studies have begun to investigate the nature of the restraints to random mixing by diffusion of surface components in mammalian sperm (31, 53, 59). Myles et al. (31) have shown that a membrane protein antigen that is localized to the posterior region of the guinea pig sperm tail is completely free to diffuse within this region anddiffuses at or near its fluid dynamic limit. Such a result is consistent with the presence of a barrier to interregional diffusion or with lateral segregation of antigen due to its insolubility in regions other than the posterior region of the tail. Wolf and Voglmayr (59) have measured the regionalization and diffusion of an exogenously added fluorescent lipid analogue on testicular and ejaculated ram spermatozoa. While this analogue stained all
regions of the sperm, both its distribution and diffusion were regionalized. At least some of the analogue was free to exchange between regions, and both distribution and diffusion changed with epididymal maturation. These experiments demonstrate that the sperm plasma membrane cannot be treated as having a single bulk membrane fluidity, but rather is laterally segregated into large scale interactive domains.

In the present paper, we consider the distribution and diffusion of a maturation-dependent membrane antigen on ram sperm. This antigen, which is a protein or glycoprotein with an $M_r = 30,000$, has been probed using a monoclonal antibody denoted ESA152. The antigen is not detectable on the surface of immature testicular sperm. It is present on all regions of the surface of ejaculated sperm, but tends to concentrate on the posterior region of the head.

The unusual distribution of ESA152 over the entire sperm surface provides us with the opportunity to extend the work of Wolf and Voglmayr (59) to a membrane protein, and to compare results with those of Myles et al. (31) for PT-1, which is highly restricted in its distribution. Like PT-1, ESA152 shows lipid-like diffusion coefficients on all regions. Unlike PT-1, significant immobile fractions are observed on all regions. Both diffusion coefficient and mobile fraction vary significantly over the surface. Exposure to bivalent antibody induces a redistribution of the antigen which excludes it from the posterior region of the head. This redistribution is associated with striking and distinct shifts in the diffusibilities of the antigen on the midpiece and head.

Materials and Methods

Sperm

Sperm were collected as described below from Shropshire rams aged 4-6 yr. These animals were maintained in a controlled environment (12 h of light/day at 15°C). Ejaculated spermatozoa and seminal plasma were obtained by electrical stimulation using the bipolar electrode described by Blackshaw (6). To avoid temperature shock, semen was collected and stored in a receptacle maintained at 34°C. Testicular sperm and rete testis fluid were collected through a catheter inserted into the rete testis as described by Voglmayr et al. (49). The free end of the catheter was placed into a receptacle attached to the anterior surface of the scrotum.

Immediately after collection, sperm were washed three times in phosphate-buffered saline (PBS) pH 7.4 that contained 5 mM glucose (PBS) by centrifugation at 500 g for 10 min. Spermatozoa can be maintained under these conditions at 34°C for 3 h without significant diminution of glycolytic activity (41).

Production of Hybridoma ESA152

Immunization. Ejaculated spermatozoa were washed three times in Krebs-Ringer-phosphate buffer and resuspended in 20 vol of the same buffer. The suspension was mixed with an equal volume of Freund's complete adjuvant, and a portion of the emulsion that contained $5 \times 10^7$ spermatozoa was injected subcutaneously into a male BALB/c mouse (Charles River Breeding Laboratories, Inc., Wilmington, MA). A similar subcutaneous injection was given 2 wk later. 2 mo after the initial immunization, the mouse was given an intraperitoneal booster injection that consisted of $2 \times 10^7$ spermatozoa in the adjuvant.

Fusion. NS1 mouse myeloma cells (P-3-NS1/1Ag4-1 [24], obtained from Dr. George S. Bloom, University of Texas, Dallas) were cultured in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 20% fetal bovine serum (HyClone Laboratories, Logan, UT) and 0.25% glucose, 1 mM sodium pyruvate, 1% 100X non-essential amino acids, and 100 µg/ml gentamicin (complete culture medium) (see references 17 and 61). Spleen cells prepared from the hyperimmunized mouse 5 d after the final booster injection were mixed with NS1 cells at a spleen cell/myeloma cell ratio of 4:1. The cells were pelleted and induced to fuse by addition of 42% (wt/vol) polyethylene glycol 3350 (Cat. No. U221, J. T. Baker Co., Sanford, ME) in 15% (vol/vol) dimethyl sulfoxide (spectrophotometric grade).

Selection and Screening. The fusion products were dispensed to 2.0-cm² wells of cluster plates to which mouse peritoneal macrophages had been allowed to attach during an overnight preincubation (14). Hybridomas were selected as the survivors of growth for 9 d in HAT medium (complete culture medium that contained 0.136 mg/ml hypoxanthine, 0.19 µg/ml aminopterin, and 3.8 µg/ml thymidine). The hybridomas were then propagated in HAT medium (complete culture medium with hypoxanthine and thymidine). Culture supernatants were screened by ELISA and indirect immunofluorescence for the presence of antibodies against dry ram spermatozoa immobilized in flat-bottom 96-well microtiter plates or on multi-well printed microscope slides (Carlson Scientific Inc., Peotone, IL). In the ELISA, sperm-associated hybridoma anti-

w Copyright 2023 Wolters Kluwer. All rights reserved. Print ISSN: 0039-7857 Online ISSN: 1524-4576

Labeling of Sperm for FPR Measurements

Washed sperm were diluted to 4 x 10⁷ cells/ml in PBS with 0.5% BSA and 0.1% NaN₃. 100 µl of sperm were incubated with either 400 µl of ESA152 or with 10 µl of the fluorescein-F₆ fragment for 15 min at room temperature. The suspension was overlayed on PBS with 0.1% NaN₃ and 5% BSA (for the intact ESA152) or 10% BSA (for the fluorescein-F₆ fragment). This was centrifuged at 400 µm for 10 min. The pellet was resuspended in PBS with 0.1% NaN₃ and 0.5% BSA. To samples labeled with ESA152, 10 µl of the rhodamine F₆ fragment of goat anti-mouse IgG was added, and the solution was stirred in the dark for 4 h at room temperature. The sample was dialyzed extensively against PBS.

A similar procedure was used to conjugate a F₆ fragment of a goat anti-mouse IgG (Cappel Laboratories) with lissamine rhodamine sulfonyl chloride (Molecular Probes, Eugene, OR).

Labeling of Sperm for Photomicroscopy

Printed microscope slides with 6-mm wells were treated twice with MICRO Laboratory Cleaner (International Products Corp., Trenton, NJ) at 90°C and
Fluorescence photomicrographs showing ESA152 staining patterns. Specific details of staining are given in the text (a). Sperm fixed in 5% glutaraldehyde and then labeled with intact ESA152 antibody followed by a rhodaminated rabbit anti--mouse IgG. Similar staining was observed in unfixed cells labeled directly with a fluorescein-labeled F_a fragment of ESA152. ESA152 is seen on all regions of the sperm surface, being most intense on the posterior region of the head (b). Unfixed sperm labeled with intact ESA152 followed by a rhodamine-labeled F_a fragment of a goat anti--mouse IgG. Cross-linking by first antibody results in a redistribution of antigen, excluding it from the posterior region of the head (c). Unfixed sperm labeled with intact ESA152 followed by a rhodamine-labeled rabbit anti--mouse IgG. In addition to the redistribution observed in b, intact second antibody leads to patching of the antigen. The particular samples in a and c were plated on poly-D-lysine as described, whereas b was labeled as described for FPR measurements. Bar, 10 μm.
method of Bligh and Dyer (7), the antigen was recovered at the interface. When the interface material was solubilized in SDS and fractionated electrophoretically in 10–20% polyacrylamide gradient gels (28), the antigen was detected by immunoblotting (21) at a position that corresponded to apparent Mr ~30,000. This zone of reactivity was eliminated by treatment with pronase or protease K in SDS before electrophoresis. These observations imply that, although the ESA152 antigen has amphipathic properties akin to lipids, it is a protein or glycoprotein. Further characterization of this interesting plasma membrane antigen is in progress, and these results will be presented separately in greater detail.

Results

Distribution of ESA152 on Ram Sperm Surfaces

Fig. 1a shows the fluorescence staining pattern of ejaculated ram spermatozoa fixed in 5% glutaraldehyde and indirectly labeled with bivalent antibody. Fixed sperm were similarly stained when intact ESA152 followed by rhodamine-conjugated F(ab) fragments of an anti-mouse IgG were used. Unfixed sperm also yielded this same pattern when labeled directly with fluorescein-conjugated F(ab) fragments of ESA152 IgG. The labeling is detectable over the entire surface of ejaculated sperm, but staining is most intense on the posterior region of the head. The staining observed is specific. In the case of indirect labeling, staining does not occur when the ESA152 is replaced by normal mouse serum or omitted altogether. In the case of the direct labeling, staining with F(ab) fragment does not occur when incubation is done in the presence of excess ascites fluid that contains intact ESA152.

Fig. 1b shows the staining pattern of unfixed ejaculated sperm labeled indirectly with intact ESA152 followed by the rhodamine-conjugated F(ab) fragment of anti-mouse IgG. Similar staining occurs on sperm fixed after the addition of ESA152. The presence of bivalent ESA152 results in a redistribution of antigen such that it is much more prominent in the equatorial region of the head and excluded from the posterior region of the head where staining is otherwise strongest.

Fig. 1c shows the staining pattern of unfixed ejaculated sperm labeled with intact ESA152 followed by rhodamine-conjugated intact rabbit anti-mouse IgG. Under these conditions, the distribution of stain is similar to that observed in Fig. 1b, except that when both antibodies are bivalent there is considerable patching of the antigen–antibody complexes.

Neither the intensity nor the distribution of ESA152 staining was measurably reduced by pretreatment of sperm with proteolytic enzymes, as described above. Pre-labeling of sperm with a variety of fluorescent lectins—concanavalin A (1 mg/ml), wheat germ agglutinin (0.8 mg/ml), soybean (1 mg/ml), Dolichos biflorus (1 mg/ml), Ulex europius Agglutinin 1 (1 mg/ml), peanut agglutinin (1 mg/ml), Ricinus communis Agglutinin 1 (1 mg/ml) (Vector Laboratories)—also had no effect on ESA152 staining intensity or distribution.

FPR Measurements

Tables I and II show the results of FPR measurements of the lateral diffusion of ESA152 on the different regions of ejaculated ram sperm labeled either directly, using the fluorescein-conjugated F(ab) fragment of ESA152, or indirectly, using intact antibody followed by rhodamine-conjugated F(ab) fragments of a goat anti-mouse IgG. Table III shows by Student’s t test the interregional comparisons as well as comparison between monovalent and intact ESA152 measurements. Where the monovalent label is used ~50% of the antigen is free to diffuse on all regions, except for the midpiece where only 28 ± 3% is free to diffuse. The diffusion rate is the same ~1 × 10⁻⁹ cm²/s on the two regions of the head, but it is faster on the midpiece (~7.0 × 10⁻⁹ cm²/s) and tail (~2.6 × 10⁻⁹ cm²/s) (see Table I). Thus, diffusion of ESA152 differs on the morphologically distinct regions of the sperm surface. Use of intact ESA152 antibody alters the diffusion as well as the distribution of the antigen. Most significant are: an increase in both the extent (to 61 ± 2%) and rate (to ~4 × 10⁻⁹ cm²/s) of diffusion on the anterior region of the head, and an striking increase in the extent of diffusion (to 50 ± 3%)
coupled with a decrease in the rate (to \( \sim 2.0 \times 10^{-9} \text{ cm}^2/\text{s} \)) on the midpiece (see Table II).

Diffusion is completely arrested when sperm are treated with 5% glutaraldehyde at pH 7.4 for 1 h on ice before labeling. Furthermore, the fluorescein-labeled \( \text{F}_{68} \) are not measurably competed off by incubation with excess intact antibody (ascites fluid) after labeling. These two controls demonstrate that the results evaluate lateral diffusion of the membrane antigen rather than hopping of the \( \text{F}_{68} \) (51) by rapid association-dissociation at the surface.

Discussion

The observation that ESA152 is present over the entire surface of ejaculated ram sperm, puts us in a position to extend the studies of Wolf and Voglmayr (59) to a membrane protein, and invites comparison with the work of Myles et al. (31) on a highly regionalized guinea pig sperm membrane protein, PT-1. Our diffusion measurements show that, as was the case for the lipid analogue, \( \text{C}_{16} \text{di-} \) (59), ESA152 exhibits different diffusibility (both D and \%R) between the morphologically distinct surface regions. The diffusion rates for ESA152 are in the range \( 10^{-9} - 10^{-10} \text{ cm}^2/\text{s} \). Significant nondiffusing fractions were observed on all surface regions. On other cell types (for review see reference 35) membrane proteins show diffusion rates \( \leq 10^{-10} \text{ cm}^2/\text{s} \) with significant immobile fractions, while lipids show diffusion rates of \( 10^{-3} - 10^{-9} \text{ cm}^2/\text{s} \) with complete diffusion. Fluid dynamic calculations of membrane protein diffusion do not predict lipid fractions and predict lipid-like diffusion rates (36, 49, 50). Thus, in most cell plasma membranes, membrane protein diffusion is not lipid fluidity limited. In some cases, disruption of membrane cell contact by blebbing causes membrane proteins to diffuse at this limit and to diffuse completely (44, 60). Thus other factors, possibly interactions with cytoskeletons and/or exoskeletons, control membrane protein diffusion. ESA152 is similar to PT-1 in that its diffusion rate is at or near this fluid dynamic limit. It is dissimilar in that it exhibits large nondiffusing fractions. Further characterization of this antigen and the nature of the epitope recognized may enable us to biochemically distinguish the diffusible and nondiffusible fractions.

The redistributions and alterations in diffusibility induced by intact ESA152 are interesting for a number of reasons. Redistribution of antigen from the posterior head to the anterior head and/or midpiece is reminiscent of ligand-induced capping (41, 45). Two differences however, must be considered. First, this redistribution occurs in the presence of azide, while capping, in general, does not. Second, when the second antibody is monovalent the fluorescence redistribution occurs without first patching. Such is uncommon in capping phenomena. A notable exception to this is capping of the artificial lipopolysaccharide stearoyl dextran on T lymphocytes (57). One can imagine several mechanisms that would lead to such redistribution in the absence of metabolic energy and patching, such as: a breakdown of an interregional barrier, lateral phase segregations induced by cross-linking of a glycolipid or glycoprotein component, and cytoskeletal rearrangements induced by cross-linking a surface receptor. This induced redistribution is probably most significant phenomenologically, in that it demonstrates that a stage-specific surface component, potentially with a receptor role, can be redistributed upon interaction with a ligand.

It is also interesting to note that unlike previously studied capping systems, where capping results in nearly complete immobilization of receptor (10, 54, 56), ESA152 after redistribution from the posterior head diffuses more rapidly and completely on both the anterior region of the head and the midpiece. Evidently, in this case, redistribution does not require immobilization.

These studies raise a number of questions about the ESA152 antigen, its diffusion, and regionalization which we are currently pursuing: (a) Since ESA152 is present on the surface of mature spermatozoa, but wholly absent from testicular spermatozoa, it is reasonable to ask whether it is secreted by the epithelia of the epididymis, and inserted from the luminal fluid of the epididymis (9, 47, 48). (b) Does the mechanism of its appearance account for its relatively high hydrophobicity and lipid-like diffusion rates? (c) Do the diffusible and nondiffusible fractions represent structurally distinct populations of ESA152? (d) Are there natural conditions that result in the redistribution of ESA152, such as hyperactivation, the acrosome reaction, or capacitation? (e) Do arrays of intramembranous particles by virtue of particle proximity induce anisotropies in diffusion within a region or restrictions to diffusion between regions? (f) Does antibody-induced cross-linking or redistribution of ESA152 affect physical properties of the membrane such as the diffusion of lipid analogues or intramembranous particle distribution or physiological properties of the sperm such as flagellar wave pattern or acrosomal fusion capacity?

Underlying these questions about ESA152 distribution and diffusion are two questions common to all membranes and membrane components: what is the physical basis for immobilization of a membrane component?; and, what is the physical relationship between mobile and immobile fractions? The authors wish to thank Dr. John McCracken, Larry Bavard, Stanley Kott, and David C. Bartolletti for technical assistance in performing these experiments. We are grateful to Dr. Stephen M. King for performing the immunoblotting analysis of extracted membrane proteins, and to Ms. Margaret Moynihan and Dr. Clare O'Connor for critical reading of the manuscript.

This work was supported in part by National Institutes of Health grants HD 17377 (D. E. Wolf), HD 09356 (J. K. Voglmayr), and CA 12708 (G. Fairbanks), and by a private grant from the A. W. Mellon Foundation to the Worcester Foundation.

Received for publication 1 April 1985, and in revised form 29 January 1986.

References

1. Axelrod, D., D. E. Koppel, J. Schlesinger, E. Elson, and W. W. Webb. 1976. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. Biophys. J. 16:1055-1069.
2. Axelrod, D., P. Ravdin, D. E. Koppel, J. Schlesinger, W. W. Webb, E. L. Elson, and T. R. Podoski. 1976. Lateral motion of fluorescently labeled acetylcholine receptors in membranes of developing muscle fibers. Proc. Natl. Acad. Sci. USA. 73:4594-4598.
3. Barisas, B. F., and M. D. Leather. 1979. Fluorescence photobleaching recovery measurements of protein absolute diffusion constants. Biophys. Chem. 10:221-229.
4. Bearer, E. L., and D. S. Friend. 1982. Modifications of anionic lipid domains preceding membrane fusion in guinea pig sperm. J. Cell Biol. 92:604-615.
5. Bevington, P. R. 1969. Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill, Inc., New York. 204-246.
6. Blackshaw, A. W. 1954. A bipolar electrode for the production of ejaculation in sheep. Aust. Vet. J. 30:249-250.
7. Bligh, E. G., and W. G. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.
membranes. *Proc. Natl. Acad. Sci. USA.* 72:3111-3113.
37. Schlegel, R. A., B. M. Phelps, A. Waggoner, L. Terada, and P. Williamson. 1980. Binding of mercaptoacetic 540 to normal and leukemic erythrocytoid cells. Cell 20:321-328.
38. Schmell, E. D., B. J. Gulyas, L. C. Yvan, and F. T. Sugat. 1982. Identification of mammalian sperm surface antigens. II. Characterization of an acrosomal cap protein and a tail protein using monoclonal anti-mouse sperm antibodies. *J. Reprod. Immunol.* 5:121-134.
39. Schmell, E. D., L. C. Yvan, B. J. Gulyas, and J. T. August. 1982. Identification of mammalian sperm surface antigens. I. Production of monoclonal anti-mouse sperm antibodies. *Fertil. Steril.* 37:249-257.
40. Schneider, S. B., and W. W. Webb. 1981. Measurement of submicron laser-beam radii. *Appl. Opt.* 20:1382-1388.
41. Schreiner, G. F., and E. R. Unanue. 1976. Membrane and cytoplasmic changes in B lymphocytes induced by ligand-surface immunoglobulins interactions. *Adv. Immunol.* 24:383-411.
42. Sharon, J., S. L. Morrison, and E. A. Kabat. 1979. Detection of specific hybridoma clones by replica immunoadsorption of their secreted antibodies. *Proc. Natl. Acad. Sci. USA.* 76:1420-1424.
43. Steuchi, L. A. 1974. Structure and function of intracellular junction. *Int. Rev. CytoL.* 39:191-283.
44. Tank, D. W., E.-S. Wu, and W. W. Webb. 1982. Enhanced molecular diffusibility in muscle membrane blebs: release of lateral constraints. *J. Cell Biol.* 92:207-212.
45. Taylor, R. B., W. H. Dufus, M. C. Raff, and S. DePetrulis. 1971. Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nature New Biol.* 233:225-227.
46. Tung, K. S. K. 1977. The nature of antigens and pathogenetic mechanisms in autoimmunity to sperm. In *Immunobiology of Gametes.* M. Edidin and J. H. Johnson, editors. Cambridge University Press, New York. 73-84.
47. Tunge, M., and J. S. Weinstaire. 1973. Membrane ultrastructure at the acrosomal cap and the equatorial segment of the mouse spermatozoon. In *Evolutionary Development of the Spermatozoon.* D. W. Fawcett and J. M. Bedford, editors. Ulan & Schwarzenberg, Baltimore. 177-186.
48. Ulven, C. A. 1971. Appearances and partitioning of plasma membrane antigens during mouse spermatoogenesis. In *The Spermatozoon.* D. W. Fawcett and J. M. Bedford, editors. Ulan & Schwarzenberg, Baltimore. 177-186.
49. Vaz, J. H. C., F. Goudsald-Zakulduo, and K. Jacobson. 1984. Lateral diffusion of lipids and proteins in bilayer membranes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 174(2):199-207.
50. Voglmayr, K. J., N. A. Musto, S. K. Saksena, P. C. Brown-Woodman, P. B. Birch, and I. G. White. 1977. Characteristics of semen collected from the cauda epididymis of conscious rams. *J. Reprod. Fertil.* 49:245-251.
51. Voglmayr, J. K., T. W. Scott, B. P. Setchell, and G. H. Whaites. 1967. Metabolism of testicular spermatozoa and charactersistics of testicular fluid collected from conscious rams. *J. Reprod. Fertil.* 14:87-99.
52. Var, W. H. C., F. Goudsald-Zakulduo, and K. Jacobson. 1984. Lateral diffusion of lipids and proteins in bilayer membranes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 174(2):199-207.
53. Wolt, D. E., E.-S. Wu, and W. W. Webb. 1982. Enhanced molecular diffusibility in muscle membrane blebs: release of lateral constraints. *J. Cell Biol.* 92:207-212.
54. Wolf, D. E., P. Henkart, and W. W. Webb. 1981. Measurement of submicron laser-beam radii. *Appl. Opt.* 20:1382-1388.
55. Wolf, D. E., and J. K. Voglmayr. 1984. Diffusion and regionalization in mouse sperm plasma membranes. *Proc. Natl. Acad. Sci. USA.* 81:3893-3904.
56. Wolf, D. E., W. H. C. Var, and S. Ishijima. Changes in sperm plasma membrane lipid diffusibility after hyperactivation during in vitro capacitation in the mouse. *J. Cell Biol.* 102:1372-1377.
57. Wolf, D. E., P. Henkart, and W. W. Webb. 1980. Diffusion, patching and capping of stauroylated dextran of T37 cell plasma membranes. *Biochemistry.* 19:3893-3904.
58. Wolf, D. E., W. H. C. Var, and S. Ishijima. Changes in sperm plasma membrane lipid diffusibility after hyperactivation during in vitro capacitation in the mouse. *J. Cell Biol.* 102:1372-1377.
59. Wolf, D. E., P. Henkart, and W. W. Webb. 1981. Diffusion, patching and capping of stauroylated dextran of T37 cell plasma membranes. *Biochemistry.* 19:3893-3904.
60. Wolf, D. E., W. H. C. Var, and S. Ishijima. Changes in sperm plasma membrane lipid diffusibility after hyperactivation during in vitro capacitation in the mouse. *J. Cell Biol.* 102:1372-1377.
61. Wolf, D. E., P. Henkart, and W. W. Webb. 1980. Diffusion, patching and capping of stauroylated dextran of T37 cell plasma membranes. *Biochemistry.* 19:3893-3904.
62. Wolf, D. E., W. H. C. Var, and S. Ishijima. Changes in sperm plasma membrane lipid diffusibility after hyperactivation during in vitro capacitation in the mouse. *J. Cell Biol.* 102:1372-1377.
63. Wolf, D. E., P. Henkart, and W. W. Webb. 1981. Diffusion, patching and capping of stauroylated dextran of T37 cell plasma membranes. *Biochemistry.* 19:3893-3904.
64. Wolf, D. E., W. H. C. Var, and S. Ishijima. Changes in sperm plasma membrane lipid diffusibility after hyperactivation during in vitro capacitation in the mouse. *J. Cell Biol.* 102:1372-1377.
65. Wolf, D. E., P. Henkart, and W. W. Webb. 1980. Diffusion, patching and capping of stauroylated dextran of T37 cell plasma membranes. *Biochemistry.* 19:3893-3904.
66. Wolf, D. E., W. H. C. Var, and S. Ishijima. Changes in sperm plasma membrane lipid diffusibility after hyperactivation during in vitro capacitation in the mouse. *J. Cell Biol.* 102:1372-1377.
67. Wolf, D. E., P. Henkart, and W. W. Webb. 1980. Diffusion, patching and capping of stauroylated dextran of T37 cell plasma membranes. *Biochemistry.* 19:3893-3904.