

Sperm Receptors and Fertilization in Mammals

PAUL M. WASSARMAN, PH.D.

Abstract

During fertilization in mammals, sperm must first bind in a species-specific manner to the egg's thick extracellular coat, the zona pellucida. They then undergo a form of cellular exocytosis, the acrosome reaction. ZP3, one of three zona pellucida glycoproteins, serves as a structural glycoprotein, a sperm receptor, and an acrosome reaction-inducer. The latter two functions are mediated, at least in part, by ZP3 oligosaccharides. ZP3 is unique to mammalian eggs, from mice to humans, although related glycoproteins are found in vitelline envelopes of virtually all non-mammalian eggs, from fish to birds. Furthermore, the "zona domain" of zona pellucida glycoproteins, a characteristic ~260 amino acid-long region, is present in many (glyco)proteins with various functions in both vertebrates and invertebrates. It is tempting to suggest that egg-coat glycoproteins have evolved from solely structural components of certain non-mammalian egg vitelline envelopes into sperm receptors and acrosome reaction-inducers of the mammalian egg zona pellucida.

Key Words: Sperm receptors, fertilization, ZP3 glycoprotein, acrosome reaction.

Introduction

INFERTILITY AFFLICTS tens of millions of adult men and women worldwide. Although not recognized as a disease, it can be an extremely unpleasant burden for those individuals and couples affected by it. While *in vitro* fertilization (IVF) offers a means of circumventing many causes of infertility, it is not a universal solution to the problem, since there are numerous causes of infertility.

For more than twenty years, my laboratory has focused its efforts on one particular aspect of fertilization in mammals, the species-specific binding of free-swimming sperm to ovulated eggs. The failure of sperm to bind to ovulated eggs is frequently a cause of infertility among mammals. Our research is concerned primarily with the thick extracellular coat that

surrounds all mammalian eggs, the zona pellucida (ZP). During the fertilization process, sperm must first bind to and then penetrate the ZP in order to reach and fuse with the egg plasma membrane (Fig. 1). In essence, the ZP serves as a "gate-keeper," to regulate sperm



Fig. 1. Binding of sperm to the egg ZP. Light photomicrograph (Nomarski differential interference contrast) of mouse sperm bound to the ZP of an unfertilized mouse egg *in vitro*. (magnification ~600X).

Address correspondence to Paul Wassarman, Ph.D., Department of Biochemistry and Molecular Biology, Box 1020, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029-6574.

Presented as a Dean's Lecture at the Mount Sinai School of Medicine, New York, NY, on February 28, 2001 and updated as of June 2001.

binding. In general, the ZP permits binding of sperm only when a sperm and an egg come from the same species ("species-specific binding"). Furthermore, once an egg has been fertilized, changes in the ZP eliminate binding of sperm, as part of the block to polyspermy. These and other features of the egg ZP strongly suggest that the ZP contains receptors ("sperm receptors") that need to be recognized by free-swimming sperm in order for them to bind to ovulated eggs in a species-specific manner (1, 2).

Twenty years ago it was already known that the mouse egg ZP possesses a sperm-receptor-like activity (3). For example, brief incubation of solubilized mouse egg ZP with mouse sperm prevented the sperm from binding to ovulated eggs. This was attributed to binding of sperm receptors present in the solubilized ZP preparations to these sperm, thereby inhibiting sperm from binding to eggs. On the other hand, solubilized ZP from either fertilized mouse eggs or preimplantation embryos had no effect on sperm binding. These, as well as other observations, prompted us, in 1977, to attempt to isolate the mouse sperm receptor and to characterize it. The following account summarizes our progress to date and suggests some areas of future research interest.

Fertilization in Mice

The final steps of mammalian oogenesis and spermatogenesis prepare eggs and sperm, respectively, for fertilization. During ovulation, fully grown oocytes from antral follicles undergo meiotic maturation and become unfertilized eggs prepared to interact with sperm. Similarly, following deposition into and migration up the female reproductive tract, sperm undergo capacitation, a process that enables them to bind to eggs and undergo the acrosome reaction (AR). Meiotic maturation of oocytes and capacitation of sperm set gametes off on a path that leads to either formation of a viable zygote or degeneration ("death") of the cells.

Typically, very few ovulated eggs are found in oviducts of females (mice, ~10). Similarly, relatively few sperm are found at the site of fertilization (mice, ~125) as compared to the number of sperm deposited into the female reproductive tract (mice, ~10⁷). Only a very low percentage of ejaculated sperm ever make their way to the site(s) of unfertilized eggs in the oviduct. Whether binding of sperm to eggs occurs due to a chance encounter of gametes in the oviduct or is promoted by a chemical gradi-

ent stimulus ("sperm chemotaxis"), as found with many non-mammalian species, remains to be resolved conclusively.

Among mammals, the process of union of germ cells includes several steps that take place in an unvarying order. It begins in the oviduct with binding of free-swimming sperm to the ovulated egg ZP and ends a short time later (~1–2 hr after combining gametes *in vitro*) with fusion of egg and sperm plasma membranes to form a single activated cell, the zygote. Along the way, several recognizable events take place, including the sperm AR (a form of cellular exocytosis), penetration of the egg ZP by the sperm, and the egg cortical reaction and zona reaction (Fig. 2). The latter results in alteration of the ZP (so-called secondary block to polyspermy), such that free-swimming sperm are unable to bind to the ZP of fertilized eggs or preimplantation embryos.

Does mammalian fertilization exhibit any species specificity? It is well known that interspecific hybrids of certain mammals are viable. However, evidence from *in vitro* fertilization experiments strongly suggests that there are, indeed, barriers to interspecific fertilization and that the egg ZP serves as a major barrier. The ZP can interfere with interspecific fertilization by failing to permit the initial binding of sperm to eggs, induction of the AR, or penetration of bound sperm through the extracellular coat. Although the restrictions on binding are not ab-

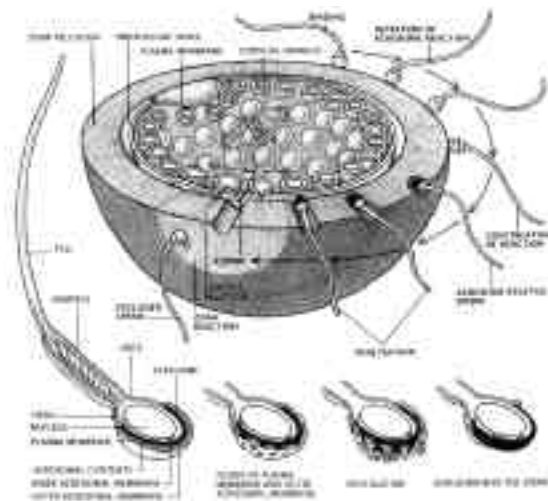


Fig. 2. Diagrammatic representation of the fertilization pathway in mice. Depicted are binding of acrosome-intact sperm to the unfertilized egg ZP; initiation of the acrosome reaction; penetration of the egg ZP by acrosome-reacted sperm; fusion of sperm and egg plasma membranes to produce a zygote; and initiation of the cortical and zona reactions.

solute, they provide for a relatively high degree of species-specific fertilization *in vitro*. Notably, removal of the ZP from unfertilized mammalian eggs, thereby exposing egg plasma membrane directly to acrosome-reacted sperm, eliminates the barrier to interspecific fertilization *in vitro* for many, but not all, mammals.

Nature of the Mouse Sperm Receptor, mZP3

The mouse sperm receptor is an ~83 kDa M_r ZP glycoprotein, called mZP3 (4–6). It is one of three glycoproteins, mZP1–3, that constitute the egg ZP (Fig. 3) (7). mZP3 consists of a polypeptide backbone to which asparagine- (N-) linked and serine/threonine- (O-) linked oligosaccharides are covalently attached. mZP3 polypeptide is synthesized as a ~44 kDa M_r species (424 amino acids); however, a signal sequence at its N-terminus (22 amino acids) and a fragment at its C-terminus (71 amino acids) are removed prior to incorporation of nascent mZP3 into the ZP (8–10). Approximately 260 amino acids of mZP3, which includes eight conserved cysteine residues, constitute a so-called “zona domain” (11), and this domain is present in mZP1 and mZP2 as well. A zona domain is present in all ZP glycoproteins, from mice to human beings, and is present at the C-terminus of many other (glyco)proteins found in both vertebrates and invertebrates

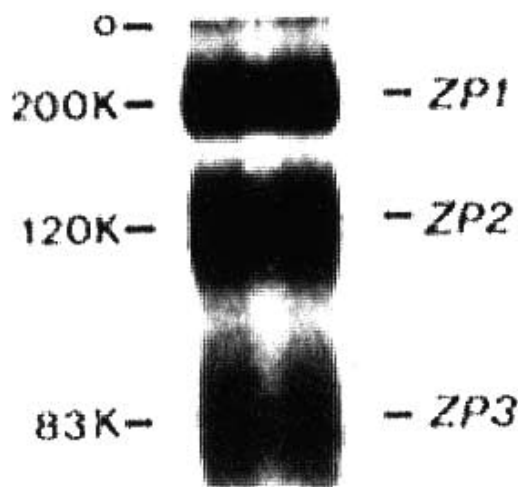


Fig. 3. Electrophoretic separation of mZP1, mZP2, and mZP3. Shown are the positions and molecular weights of radiolabeled ZP glycoproteins following one-dimensional sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions and autoradiography. Note the breadth of the individual bands, reflecting heterogeneous—and O-linked glycosylation of unique polypeptides.

(e.g., tectorin- β , uromodulin, ebnerin, cuticlin [*C. elegans*], and dumpy [*Drosophila*]) (12).

Of the three glycoproteins in the mouse egg ZP, only purified mZP3 binds exclusively to heads of acrosome-intact sperm, thereby preventing sperm from binding to ovulated eggs *in vitro* (4, 5, 13). Even at nanomolar concentrations, purified, unfertilized egg mZP3 is a very effective inhibitor of sperm binding (Fig. 4). On the other hand, at similar concentrations, mZP3 from fertilized eggs or early embryos has no effect on binding of sperm to eggs *in vitro*. This is consistent with the failure of free-swimming sperm to bind to the ZP of fertilized eggs and preimplantation embryos. It can be concluded from these and other observations that, as a consequence of the “zona reaction” shortly after fertilization, mZP3 is altered such that free-swimming sperm can no longer recognize and bind to the glycoprotein.

Molecular Basis of mZP3 Sperm Receptor Activity

Interestingly, the ability of mZP3 to act as a sperm receptor *in vitro* is not significantly affected by high temperatures, detergents, denat-

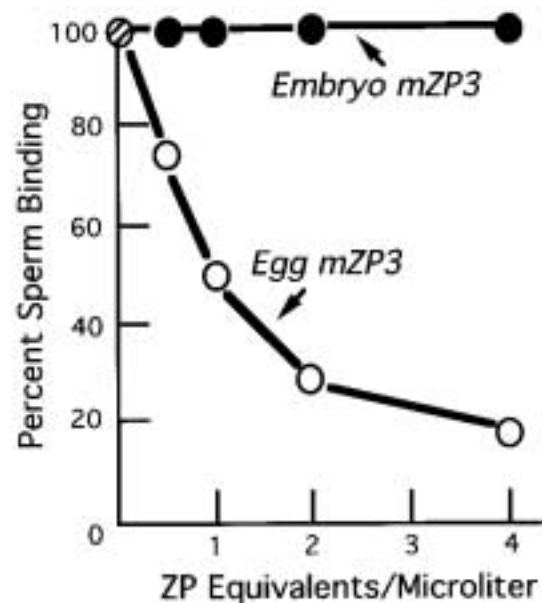


Fig. 4. Inhibition of sperm binding to eggs in the presence of purified egg mZP3 *in vitro*. Binding of mouse sperm to eggs was examined in the absence of mZP3 (hatched circle) and in the presence of either purified egg mZP3 (open circles) or two-cell embryo mZP3 (closed circles) at several concentrations. Note that egg mZP3 is a very effective inhibitor at nanomolar concentrations, whereas embryo mZP3 is inactive as an inhibitor at comparable concentrations.

urants, reducing agents, or limited proteolysis (14). Acrosome-intact sperm even bind to glass beads to which egg mZP3 is covalently linked (15). Apparently, mZP3 bioactivity is not dependent on the extent of glycosylation of its polypeptide or on sulfation and sialylation of its oligosaccharides (16). Even after extensive proteolysis of mZP3, the small glycopeptides produced retain activity as sperm receptors, although higher than usual concentrations are required (5, 17, 18). These observations suggest that mZP3 polypeptide does not play a direct role in sperm receptor function. However, there is considerable information to suggest that mZP3 oligosaccharides do play a direct role in sperm receptor function.

Chemical or enzymatic removal of all mZP3 oligosaccharides results in complete inactivation of the glycoprotein as a sperm receptor. Furthermore, O-linked oligosaccharides recovered from mZP3 by mild alkaline hydrolysis under reducing conditions (18–20) and certain O-linked-related oligosaccharides (21, 22) inhibit binding of sperm to eggs *in vitro* at micromolar concentrations. Results of some of these studies suggest that galactose, N-acetylglucosamine, and/or fucose are essential sugars for sperm binding. Collectively, these observations suggest that species-specific binding of sperm to eggs in mammals is a carbohydrate-mediated event. On the other hand, the identity of individual sugars that are recognized by sperm on mZP3 remains an unresolved and controversial issue (6).

To locate essential O-linked oligosaccharides on mZP3 polypeptide, limited proteolysis (23, 24), exon swapping (25) and site-directed mutagenesis (25, 26) were utilized. Results of such studies suggest that all sperm receptor activity of mZP3 is associated with the C-terminal half of the polypeptide. The essential oligosaccharides are present on just two of five serine residues, serine-332 and -334, in a region of polypeptide near the C-terminus, a region encoded by exon-7 of the *mZP3* gene (8 exons). For example, mutation of either serine-332 or serine-334 to a small aliphatic amino acid results in production of an inactive form of mZP3 in stably transfected cells. Interestingly, of the five serine residues, only these two are conserved from mouse to human ZP3. In this context, the numerous amino acid changes neighboring serine-332 and -334 that have occurred during evolution may impose changes in the structure of O-linked oligosaccharides added to ZP3 and, thereby, affect species specificity of sperm-egg interaction (27, 28). This is cur-

rently an active area of investigation in several laboratories.

mZP3 Is an Acrosome Reaction-Inducer

The acrosome is a large secretory vesicle that overlies the nucleus in the apical region of the sperm head (6, 29). The acrosomal membrane just underlying the plasma membrane is referred to as “outer” acrosomal membrane and that overlying the nucleus is referred to as “inner” acrosomal membrane. Morphologically, the AR is seen as multiple fusions between the outer acrosomal membrane and the plasma membrane at the anterior region of sperm head, extensive formation of hybrid membrane vesicles, and exposure of inner acrosomal membrane and acrosomal contents (Fig. 5). Only acrosome-reacted sperm (Fig. 6) can penetrate the ZP and fuse with the egg plasma membrane.

Several of the same kinds of molecules that participate in secretion by somatic cells participate in initiation of the AR (12, 30, 31). These include several signal-transducing components, including G proteins, inositol 3,4,5-triphosphate (IP₃) and IP₃ receptors, phospholipase C, Ca²⁺, and voltage-sensitive Ca²⁺ channels. For example, mZP3 stimulation of sperm activates G proteins (G_{i1}, G_{i2}, G_{q/11}), depolarizes sperm plasma membrane (from ~-60 mV to ~-30 mV), activates Ca²⁺ channels (T-type), and elevates pH (~0.3 units) and intracellular Ca²⁺ concentration (from ~150 nM to ~400 nM). Activation of a pertussis toxin-sensitive G protein complex has been attributed to aggregation of -galactosyl-

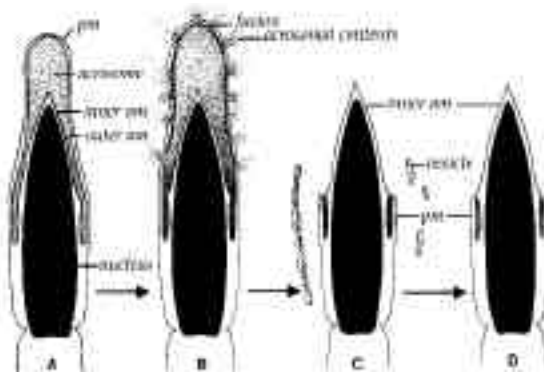


Fig. 5. Diagrammatic representation of the mammalian sperm acrosome reaction. The course of the acrosome reaction is indicated by A-D. An acrosome-intact sperm head is shown in A. In B fusion between outer acrosomal membrane and plasma membrane is indicated. Hybrid membrane vesicles, composed of plasma and outer acrosomal membrane, are shown in C and D. pm = plasma membrane; am = acrosomal membrane.

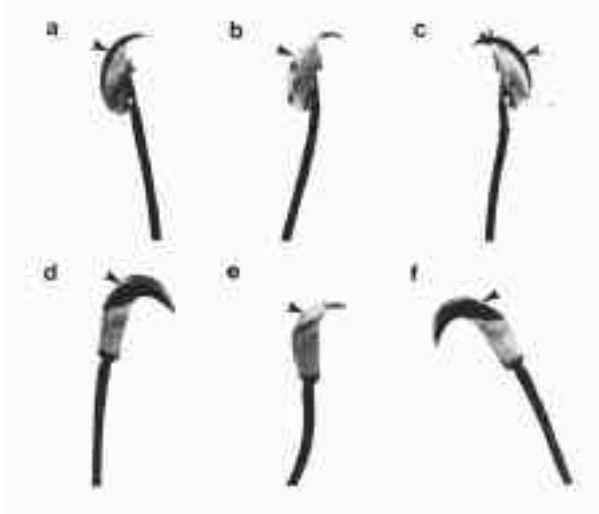


Fig. 6. Acrosome-intact and acrosome-reacted mouse and hamster sperm. Light photomicrographs of mouse (a-c) and hamster (d-f) sperm stained with Coomassie brilliant blue G-250, fixed, and examined by light microscopy. (magnification $\sim 1000\times$). Acrosome-intact (a, c, d, f) and acrosome-reacted (b, e) sperm are shown. The acrosome reaction was induced with purified egg mZP3 *in vitro*. Arrowheads indicate the position of the acrosome.

transferase on the sperm head, and Ca^{2+} entry through store-operated channels is thought to result from depletion of IP_3 -sensitive Ca^{2+} stores; both events are triggered by exposure of sperm to mZP3. Finally, in this context, results of recent studies suggest that at least two components essential for intracellular membrane fusion in somatic cells, Rab3A GTPase and SNAREs, are present in mammalian sperm and may participate in membrane fusion during the AR.

It is known that there are many different inducers of the AR (29). However, it is now generally accepted that ZP3 is the natural agonist that initiates the AR upon binding of acrosome-intact mammalian sperm to the ZP (32–34). There are now criteria that distinguish between the so-called “spontaneous” AR and the ZP3-induced AR (e.g., sensitivity to pertussis toxin). While purified mZP3 and large mZP3 glycopeptides induce sperm to acrosome-react *in vitro*, small mZP3 glycopeptides and purified mZP3 O-linked oligosaccharides bind to sperm and inhibit their binding to eggs, but do not induce the AR (5, 14). In the latter context, it has been reported that IgG cross-linking of small mZP3 glycopeptides bound to sperm can induce the sperm to acrosome-react (35). These findings suggest that induction of the AR by ZP3 probably will turn out to be dependent on multivalent interactions between ZP3 and its binding-protein at the sperm surface.

Phenotype of mZP3 Homozygous Null Mice

The three mouse ZP glycoproteins are organized in a specific fashion in the ZP (6, 36, 37). Each of the glycoproteins is synthesized, secreted, and assembled into a ZP by oocytes during their 2–3-week growth phase (8, 38, 39). The extracellular coat consists of very long filaments composed of mZP2-mZP3 dimers that are cross-linked by mZP1 (Fig. 7). The filaments exhibit a structural periodicity of ~ 15 nm that represents the positions of mZP2-mZP3 dimers along the filaments. The three glycoproteins are held together in the ZP by non-covalent bonds.

What happens when one of the ZP glycoproteins is not available for assembly of a ZP during oogenesis? The single-copy gene-encoding mZP3 consists of 8 exons (40). mZP3 null mutant mice (mZP3^{-/-}) were produced using homologous recombination in embryonic stem (ES) cells by following standard gene targeting procedures (41, 42). Female mZP3^{-/-} mice were obtained that are indistinguishable in appearance from wild-type littermates and exhibit normal growth. However, mZP3^{-/-} females are infertile and the infertility appears to be related to the complete absence of a ZP surrounding growing oocytes and ovulated eggs (Fig. 8). Examination of superovulated mZP3^{-/-} females revealed that while cumulus masses were always found in their oviducts, frequently no eggs were present within the cumulus

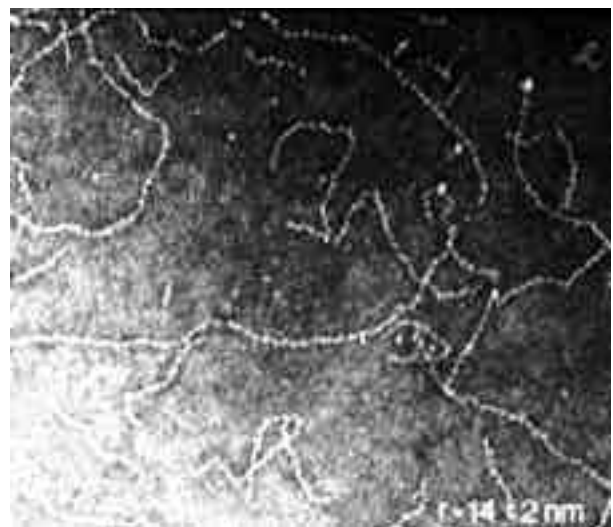


Fig. 7. The ZP is composed of long, cross-linked filaments. Shown is a transmission electron micrograph of mouse egg ZP filaments sprayed onto a grid and negatively stained. The filaments are composed of mZP2 and mZP3 and are cross-linked by mZP1. A structural repeat of 14 ± 2 nanometers along each filament is indicated.

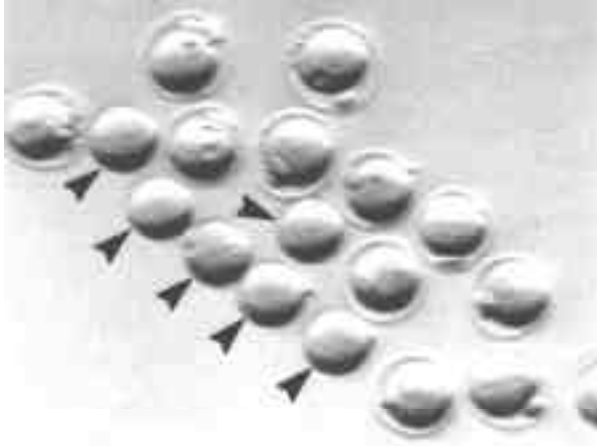


Fig. 8. Ovulated eggs obtained from mice homozygous null for *mZP3* lack a ZP. Shown are six ovulated eggs lacking a ZP recovered from *mZP3*^{-/-} mice (arrowheads) and twelve ovulated eggs which have a ZP recovered from wild-type (*mZP3*^{+/+}) mice. The light photomicrograph was taken using Nomarski differential interference contrast microscopy. (magnification ~135X).

masses. In this context, the yield of growing oocytes from ovaries of null mice is a small fraction of that obtained from ovaries of wild-type mice.

These and other observations suggest that in the absence of mZP3, a ZP cannot be assembled around growing oocytes; i.e., mZP3 plays a structural role in assembly and maintenance of the ZP. Furthermore, the absence of a ZP deleteriously affects oocyte growth, follicle development, and fertilization. It seems likely that these effects are attributable to reduced intercellular communication via gap junctions between growing oocytes and innermost follicle cells (*corona radiata*). The viscous border established by the ZP appears to be necessary to stabilize interactions between the two cell types and promote follicle development, including oocyte growth.

Final Comments

There is great diversity when it comes to the mechanism of species-specific fertilization. For example, some non-mammalian eggs lack an extracellular coat (e.g., nematode eggs) while others have a vitelline envelope and a jelly coat (e.g., echinoderm and amphibian eggs). In some species, sperm enter eggs at a particular site (micropyle) without the need for an acrosome reaction (e.g., nematodes), whereas in others sperm undergo the acrosome reaction upon binding to the jelly coat and then bind in a species-specific manner to the vitelline enve-

lope (e.g., echinoderms and amphibians). In the latter context, one might think of the ZP, which is unique to all mammalian eggs, as an amalgamation of the jelly coat and vitelline envelope of some non-mammalian eggs. The ZP is the site of species-specific sperm receptors and an inducer of the acrosome reaction.

I have presented what has been learned about the functions of ZP glycoprotein mZP3 during fertilization, with special emphasis on the role of oligosaccharides in mZP3 function. For example: mZP3 is a structural glycoprotein absolutely required for ZP assembly during oogenesis; mZP3 is a sperm receptor that supports binding of free-swimming sperm to unfertilized eggs; and mZP3 is an acrosome reaction-inducer for sperm bound to the ZP of unfertilized eggs. The sperm receptor and acrosome reaction-inducing activities of mZP3 depend on the glycoprotein's oligosaccharides. In mice, these oligosaccharides apparently are located on two serine residues located near the C-terminus of the polypeptide. This portion of the polypeptide has undergone significant changes during evolution, and it is possible that these changes result in alternative oligosaccharide structures for ZP3 from different mammalian species. The cell's rules for O-linked glycosylation of nascent proteins will have to be determined in order to answer key questions about ZP3 oligosaccharides.

Although we know a great deal about mZP3, many more questions remain. A pressing issue concerns the nature of the protein associated with plasma membrane surrounding the sperm head that recognizes and binds to mZP3 oligosaccharides (so-called, egg-binding protein). This area of research, while extremely productive, remains inconclusive (6). A second issue concerns the nature of the ZP3 oligosaccharides that are recognized by sperm. We know very little about the structure of these oligosaccharides (e.g., composition, sequence, and linkages) or their sites on ZP3 polypeptides from different species of mammalian eggs. Can these oligosaccharides account for the species-specific binding of sperm that is observed experimentally? Finally, the mechanism by which inactivation of mZP3 inactivates the sperm receptor following fertilization needs to be worked out. Preliminary evidence suggests that inactivation can be attributed to enzymatic destruction of essential mZP3 oligosaccharides (PM Wassarman, unpublished results). We hope that answers to these and other questions will be forthcoming and that such progress will be of some benefit to clinicians involved in the manipulation of human reproduction.

Acknowledgments

For more than twenty years many outstanding research assistants, graduate students, and post-doctoral fellows, at Harvard Medical School, the Roche Institute of Molecular Biology, and Mount Sinai Medical School, have contributed to the research described here. I am grateful to all of them for their scientific contributions and for the spirit they imparted to our laboratory. This research is currently supported by the NICHD (HD-35105).

References

1. Wassarman PM. The biology and chemistry of fertilization. *Science* 1987; 235:553–560.
2. Wassarman PM. Fertilization in mammals. *Sci Am* 1988; 255:78–84.
3. Gwatkin RBL. Fertilization mechanisms in man and mammals. New York: Plenum Press; 1977.
4. Bleil JD, Wassarman PM. Mammalian sperm-egg interaction: Identification of a glycoprotein in mouse egg zonae pellucidae possessing receptor activity for sperm. *Cell* 1980; 20:873–882.
5. Wassarman PM. Profile of a mammalian sperm receptor. *Development* 1990; 108:1–17.
6. Wassarman PM. Mammalian fertilization: Molecular aspects of gamete adhesion, exocytosis, and fusion. *Cell* 1999; 96:175–183.
7. Bleil JD, Wassarman PM. Structure and function of the zona pellucida: Identification and characterization of the proteins of the mouse oocyte's zona pellucida. *Dev Biol* 1980; 76:185–202.
8. Salzmann GS, Greve JM, Roller RJ, Wassarman PM. Biosynthesis of the sperm receptor during oogenesis in the mouse. *EMBO J* 1983; 2:1451–1456.
9. Litscher ES, Qi H, Wassarman PM. Mouse zona pellucida glycoproteins mZP2 and mZP3 undergo carboxy-terminal proteolytic processing in growing oocytes. *Biochemistry* 1999; 38:12280–12287.
10. Williams Z, Wassarman PM. Secretion of mouse ZP3, the sperm receptor, requires cleavage of its polypeptide at a consensus furin cleavage-site. *Biochemistry* 2001; 40:929–937.
11. Bork P, Sander C. A large domain common to sperm receptors (ZP2 and ZP3) and TGF- β type III receptor. *FEBS Lett* 1992; 300:237–240.
12. Wassarman PM, Jovine L, Litscher ES. A profile of fertilization in mammals. *Nat Cell Biol* 2001; 3:E59–E64.
13. Mortillo S, Wassarman PM. Differential binding of gold-labeled zona pellucida glycoproteins mZP2 and mZP3 to mouse sperm membrane compartments. *Development* 1991; 113:141–149.
14. Wassarman PM. Zona pellucida glycoproteins. *Annu Rev Biochem* 1988; 57:415–442.
15. Vazquez MH, Phillips DM, Wassarman PM. Interaction of mouse sperm with purified sperm receptors covalently-linked to silica beads. *J Cell Sci* 1989; 92:713–722.
16. Liu C, Litscher ES, Wassarman PM. Zona pellucida glycoprotein mZP3 bioactivity is not dependent on the extent of glycosylation of its polypeptide or on sulfation and sialylation of its oligosaccharides. *J Cell Sci* 1997; 110:745–752.
17. Florman HM, Bechtol KD, Wassarman PM. Enzymatic dissection of the functions of the mouse egg's receptor for sperm. *Dev Biol* 1984; 106:243–255.
18. Florman HM, Wassarman PM. O-Linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. *Cell* 1985; 41:313–324.
19. Bleil JD, Wassarman PM. Galactose at the non-reducing terminus of O-linked oligosaccharides of mouse egg zona pellucida glycoprotein ZP3 is essential for the glycoprotein's sperm receptor activity. *Proc Natl Acad Sci U S A* 1988; 85:6778–6782.
20. Miller DJ, Macek MB, Shur BD. Complementarity between sperm surface 1,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. *Nature* 1992; 357:589–593.
21. Litscher ES, Juntunen K, Seppo A, et al. Oligosaccharide constructs with defined structures that inhibit binding of mouse sperm to unfertilized eggs in vitro. *Biochemistry* 1995; 34:4662–4669.
22. Johnston DS, Wright WW, Shaper JH, et al. Murine sperm-zona binding, a fucosyl residue is required for a high affinity sperm-binding ligand. *J Biol Chem* 1998; 273:1888–1895.
23. Rosiere TK, Wassarman PM. Identification of a region of mouse zona pellucida glycoprotein mZP3 that possesses sperm receptor activity. *Dev Biol* 1992; 154:309–317.
24. Litscher ES, Wassarman PM. Characterization of a mouse ZP3-derived glycopeptide, gp55, that exhibits sperm receptor and acrosome reaction-inducing activity in vitro. *Biochemistry* 1996; 35:3980–3985.
25. Kinloch RA, Sakai Y, Wassarman PM. Mapping the mouse ZP3 combining-site for sperm by exon swapping and site-directed mutagenesis. *Proc Natl Acad Sci U S A* 1995; 92:263–267.
26. Chen J, Litscher ES, Wassarman PM. Inactivation of the mouse sperm receptor, mZP3, by site-directed mutagenesis of individual serine residues located at the combining-site for sperm. *Proc Natl Acad Sci U S A* 1988; 95:6193–6197.
27. Wassarman PM, Litscher ES. Sperm-egg recognition mechanisms in mammals. *Curr Top Dev Biol* 1995; 30:1–19.
28. Swanson WJ, Yang Z, Wolfner MF, Aquadro CF. Positive Darwinian selection drives the evolution of several female reproductive proteins in mammals. *Proc Natl Acad Sci USA* 2001; 98:2509–2514.
29. Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD, editors. *The physiology of reproduction*. New York: Raven Press; 1994. pp. 189–317.
30. Darszon A, Labarca P, Nishiguchi T, Espinosa F. Ion channels in sperm physiology. *Physiol Rev* 1999; 79:481–510.
31. Florman HM, Arnoult C, Kazam IG, et al. An intimate biochemistry. Egg-regulated acrosome reactions of mammalian sperm. *Adv Dev Biochem* 1999; 5:199–233.
32. Bleil JD, Wassarman PM. Sperm-egg interactions in the mouse: Sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. *Dev Biol* 1983; 95:317–324.
33. Darszon A, Liévano A, Beltran C. Ion channels: Key elements in gamete signaling. *Curr Topics Dev Biol* 1996; 34:117–167.
34. Florman HM, Arnoult C, Kazam IG, et al. A perspective on the control of mammalian fertilization by egg-activated ion channels in sperm: A tale of two channels. *Biol Reprod* 1998; 59:12–16.
35. Leyton L, Saling P. Evidence that aggregation of mouse sperm receptors by ZP3 triggers the acrosome reaction. *J Cell Biol* 1989; 108:2163–2168.
36. Greve JM, Wassarman PM. Mouse egg extracellular coat is a matrix of interconnected filaments possessing a structural repeat. *J Mol Biol* 1985; 181:253–264.
37. Wassarman PM, Mortillo S. Structure of the mouse egg extracellular coat, the zona pellucida. *Int Rev Cytol* 1991; 130:85–109.

38. Greve JM, Salzmann GS, Roller RJ, Wassarman PM. Biosynthesis of the major zona pellucida glycoprotein secreted by oocytes during mammalian oogenesis. *Cell* 1982; 31:749–759.
39. Lira SA, Kinloch RA, Mortillo S, Wassarman PM. An upstream region of the mouse ZP3 gene directs expression of firefly luciferase specifically to growing oocytes in transgenic mice. *Proc Natl Acad Sci U S A* 1990; 87:7215–7219.
40. Kinloch RA, Roller RJ, Fimiani C, et al. Primary structure of the mouse sperm receptor's polypeptide chain determined by genomic cloning. *Proc Natl Acad Sci U S A* 1988; 85:6409–6413.
41. Liu C, Litscher ES, Mortillo S, et al. Targeted disruption of the mZP3 gene results in production of eggs lacking a zona pellucida and infertility in female mice. *Proc Natl Acad Sci USA* 1996; 93:5431–5436.
42. Wassarman PM, Liu C, Chen J, et al. Ovarian development in mice bearing homozygous or heterozygous null mutations in zona pellucida glycoprotein gene mZP3. *Histol Histopathol* 1998; 13:293–300.