Perivitelline Space: Does It Play a Role in Blocking Polyspermy in Mammals?

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ABSTRACT The perivitelline space of mammalian oocytes changes in size and composition during preimplantation development. Often overlooked in the past, this space contains a hyaluronan-rich extracellular matrix prior to fertilization and a cortical granule envelope following release of the cortical granules at fertilization. The hyaluronan-containing matrix of unfertilized oocytes is well developed in some species such as opossums and humans but is scant in rodents including the hamster and mouse. The significance of the hyaluronan-rich matrix, which attaches to the plasma membrane of the oocytes, is not fully understood. However, hyaluronan, which can inhibit membrane fusion, is present in the perivitelline space (PVS) of unfertilized oocytes and must be negotiated by the fertilizing sperm. Following fertilization, the cortical granule envelope forms as the cortical granules disperse, thereby causing the PVS to increase significantly in size. Calcium is important in the dispersion of the cortical granules following exocytosis. Once formed, the cortical granule envelope in some species is about the same thickness as the zona pellucida, but it is not readily visualized unless it is stained with fluorescent probes or examined ultrastructurally after following stabilization with ruthenium red. The envelope contains proteins that remain in the PVS until the time of blastocyst hatching. Although little work has been done on the functions of the cortical granule envelope, several studies are consistent with the idea that it plays a role in blocking polyspermy. While nicotine increases polyspermy in sea urchins, its effects on polyspermy in human smokers have not been characterized, but could be addressed in human in vitro fertilization labs. Microsc. Res. Tech. 61:349–357, 2003.

WHAT IS THE PERIVITELLINE SPACE?
The perivitelline space (PVS) is a “space” between the surface of the oocyte or more specifically the oolemma and the zona pellucida, an extracellular matrix synthesized by the oocyte. The term space is a misnomer as it implies something that is empty and perhaps uninteresting. In fact, the PVS has contents that change during development and that appear to play various roles before, during, and after fertilization. The purpose of this review will be to discuss what is known about the PVS of mammals before and after fertilization and to examine the evidence that the contents of the PVS play a role in blocking polyspermy.

SIZE OF THE PVS
The PVS varies considerably in size at different times in development. The PVS around germinal vesicle containing oocytes is relatively small and difficult to visualize with light microscopy (Fig. 1A). After extrusion of the first polar body, the PVS becomes asymmetrical and enlarged around the first polar body, and it retains this appearance at the time of fertilization (Fig. 1B). After fertilization, the PVS remains asymmetrical and large around the polar bodies (Fig. 1C). As cleavage occurs, the PVS takes on a different shape as it follows the contours of the blastomeres (not shown).

FORMATION OF THE PVS
The PVS forms during oogenesis. Resting oocytes (10–15 μm) lack a zona pellucida. When oocytes in follicles begin to grow, the proteins ZP1, ZP2, and ZP3 are synthesized by the oocytes, secreted, and assembled extracellularly to form the zona pellucida (Flechon et al., 1984; Liang and Dean, 1993; Shimizu et al., 1983). Although the developing zona is initially made up of discontinuous clumps of zona matrix, these clumps eventually coalesce forming a complete enclosure around the oocyte (Zuccotti et al., 1991). Formation of the complete zona defines the area known as the PVS. The PVS exists as long as the zona exists, which is until the time of blastocyst hatching in the uterus just prior to implantation (Yanagimachi, 1994).

CONTENTS OF THE PVS BEFORE FERTILIZATION
The PVS surrounding follicular oocytes is small (Fig. 1A). The zona pellucida has been shown to be quite permeable, even to relatively large molecules (Hastings et al., 1972; Sellens and Jenkinson, 1975) and virus particles (Gwatkin, 1967). The PVS around follicular oocytes would thus contain follicular fluid that permeates through the zona from the fluid accumulated in...
the surrounding antrum of the follicle. This fluid is very similar to serum (Collins et al., 1997). It is not known if the zona filters the follicular fluid allowing only certain components to pass into the PVS.

Prior to ovulation and in response to luteinizing hormone, the cumulus cells undergo expansion by secreting and assembling an extracellular matrix that is rich in hyaluronic acid (Phillips and Dekel, 1982; Zhuo and Kimata, 2001). While this matrix has been reported to contain a number of different proteins and glycosaminoglycans (Zhuo and Kimata, 2001), hyaluronan covalently bonded to inter-alpha trypsin inhibitor appears to be the major component of the matrix (Chen et al., 1994, 1996; Hess et al., 1999). CD-44, a hyaluronan receptor has recently been reported on cumulus cells (Campbell et al., 1995; Kimura et al., 2002; Ohta et al., 1999) and by binding hyaluronan may hold the assembled matrix within the oocyte cumulus complex. The secretion of this matrix causes the cumulus cells to expand apart from each other. The cumulus matrix of unfertilized mammalian oocytes is not readily seen using light microscopy unless methods to stain it are employed. When oocyte cumulus complexes are fixed for microscopy in the presence of ruthenium red, the ECM between the cumulus cells is composed of electron dense granules and filaments (Talbot, 1984; Talbot and DiCarlantonio, 1984a). The granules are sensitive to trypsin while the filaments are digested by hyaluronidase (Talbot and DiCarlantonio, 1984b). Thin projections of the cumulus cells adjacent to the zona pellucida pass through the zona and attach via gap junctions to the surface of the oocyte (Anderson and Albertini, 1976). These projections apparently also secrete the same extracellular matrix that is present between cumulus cells into the PVS (Fig. 2A–C). Similar granules and filaments are present in the PVS of unfertilized mammalian oocytes after cumulus cell expansion, and both filaments and granules appear to attach to the oolemma (Dandekar et al., 1992, 1995; Dandekar and Talbot, 1992). Hyaluronan has also been localized ultrastructurally within the PVS and on the surface of the hamster oolemma using hyaluronidase conjugated to gold (Kan, 1990), consistent with the data obtained using ruthenium red. This granular filamentous matrix has been demonstrated in the PVS of rodents such as the hamster and mouse where it is relatively sparse (Dandekar and Talbot, 1992), in humans (Fig. 2A) and pigs where it is more abundant (Dandekar et al., 1992; Talbot, 1985), and in marsupials (Fig. 2B) and rabbits (Fig. 2C) where it is particularly well developed (Breed and Leigh, 1988; Dandekar et al., 1995; Talbot and DiCarlantonio, 1984b). Therefore, unfertilized oocytes of eutherian mammals and marsupials are characterized by a relatively small PVS that contains hyaluronan and probably the other proteins found in the matrix between cumulus cells.

The content of the PVS is modified after passage of the oocyte cumulus complex into the oviduct where the

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Fig. 1. Light micrographs showing the PVS (arrow) around a germinal vesicle stage oocyte (A), mature unfertilized oocyte (B), and a recently fertilized oocyte (C). The space is initially small, but becomes asymmetric and increases around the polar body after its extrusion.
zona is bathed in oviductal fluid. All mammalian oviducts except perhaps rats (Arias et al., 1994) and mares (Buhi et al., 1996), synthesize and secrete oviduct secretory glycoprotein (OGP) in their non-ciliated cells (Buhi, 2002). This protein has been referred to by various names including OSP, EAP, EGP, oviductin, MUC-9, and GP215, and it is synthesized only by the oviduct (Buhi, 2002). A number of studies have shown that OGP passes into the zona pellucida and PVS and that it accumulates in both of these regions prior to fertilization (Buhi et al., 2000). It is interesting that passage of OGP through the zona may be mainly one-directional. In mice, OGP (GP215) appears to be selectively sequestered in the PVS (Kapur and Johnson, 1986). For example, non-immune secondary antibodies used as controls gain access to the PVS but are readily removed by brief washes in antibody free medium (Kapur and Johnson, 1986). Moreover, mouse serum albumin can readily penetrate the zona and is retained by the zona after removal from the oocyte, but albumin is not found in the PVS of zona intact oocytes, even though albumin (~66 kDa) is a much smaller protein than OGP (~215 kDa) (Kapur and Johnson, 1986). OGP, however, once in the PVS is retained there and can be demonstrated using immunocytochemical methods. These data suggest that entry and retention of some proteins within the PVS is selective. The granular-filamentous matrix in the PVS space of unfertilized oocytes is clearly retained as is the oviductal OGP. OGP has been suggested to play numerous roles in fertilization. In the PVS, it may aid in fertilization by increasing fertilization rate and decreasing polyspermy (Kouba et al., 2000), although this latter point has not been confirmed in all studies (McCaulley et al., 2001).

WHAT IS THE SIGNIFICANCE OF THE MATRIX IN THE PVS OF UNFERTILIZED OOCYTES?

It is not known yet if the granular-filamentous matrix in the PVS of unfertilized oocytes plays any role in oogenesis or fertilization. The matrix is rich in hyaluronan, which would tend to draw water into the space. Indeed the size of the PVS appears to be related to the amount of granular-filamentous matrix present. For example, in opossums, which have an abundant matrix in the PVS, the space before fertilization is relatively large (Talbot and DiCarlantonio, 1984b), while in rodents that have a very modest matrix the space is small (Dandekar and Talbot, 1992). The size of the space before fertilization could affect how the fertilizing sperm approaches the oolemma. In mammals, gamete membrane fusion occurs between the sperm’s plasma membrane overlying the equatorial segment of the acrosome and the tips of the oocyte microvilli (Bedford and Cooper, 1978; Talbot and Chacon, 1982). For example, in rodents a sperm passing through the zona and entering the PVS would tend to have its head forced sideways so that the fusogenic part of the sperm’s plasma membrane overlying the equatorial segment of the acrosome and the tips of the oocyte microvilli, thereby facilitating fusion (Barros and Franklin, 1968; Yanagimachi and Noda, 1970).

Little work has been done to determine what effect hyaluronan in the PVS would have on gamete membrane fusion. In ultrastructural studies, hyaluronan appears to be anchored to the oolemma as well as present in the PVS (Dandekar et al., 1992, 1992; Kan, 1990). Somatic cell fusion can be inhibited by hyaluro-
nan (Kujawa et al., 1986), and it is possible that the presence of hyaluronan in the PVS and on the oolemma could impede gamete membrane fusion. Even after acrosome loss, sperm carry substantial amounts of hyaluronidase that become redistributed to the inner acrosomal membrane (Cowan et al., 1991, 1986) where it could readily encounter hyaluronan in the PVS. Thus, it is plausible that sperm hyaluronidase functions in degrading hyaluronan in the PVS and on the oolemma, thereby facilitating gamete membrane fusion. This could be particularly important in species having a well-developed hyaluronan-containing matrix in the PVS. It is interesting that during conventional in vitro fertilization of porcine oocytes, exogenous hyaluronan increased monospermy (Suzuki et al., 2000), which could be due to impeding gamete membrane fusion. If the cortical reaction releases an inhibitor(s) of hyaluronidase, then the presence of undegraded hyaluronan in the PVS and on the oolemma could block fusion of additional sperm. This idea, however, has not yet been investigated experimentally, and it is complicated by the observation that mammalian sperm appear to have more than one form of hyaluronidase (Baba et al., 2002).

**THE PVS AFTER FERTILIZATION**

After fertilization, the PVS of rodents increases significantly in size (Fig. 1B). Fertilization initiates the cortical reaction resulting in release of the cortical granule contents into the PVS (Cherr and Ducibella, 1990; Hoodbhoy and Talbot, 1994). Some contents are thought to diffuse through the zona pellucida and set up the zona reaction as a block to polyspermy (Cherr and Ducibella, 1990; Cran and Esper, 1990). Several proteinases have been identified that are thought to fulfill this role by modifying the zona (Gwatkin et al., 1973) or more specifically the zona protein ZP2 (Moller and Wassarman, 1989) and preventing further binding of sperm to the zona. In addition, N-acetylglucosaminidase is present in mouse cortical granules and has been shown experimentally to cause the loss in sperm-binding activity leading to the zona block to polyspermy (Miller et al., 1993). Finally, a 32-kD protein was recently shown to be a cortical granule protein. The function of this protein is not yet established; however, in in vitro experiments, a monoclonal antibody to this protein did not affect fertilization or polyspermy (Gross et al., 2000).

It has also become apparent from electron microscopic and confocal scanning laser microscopic examination of fertilized rodent and human oocytes that not all cortical granule components diffuse into the zona pellucida. Some of the cortical granule contents dehisce and remain in the PVS to form a new extracellular matrix, referred to as the cortical granule envelope (Fig. 3A–C) (Dandekar and Talbot, 1992). At the TEM level, this envelope is made of numerous electron dense granules, distinct in size and texture from those found around unfertilized oocytes (Dandekar et al., 1992, 1995; Dandekar and Talbot, 1992). Initially upon release, the granule contents remain aggregated (Fig. 3A) even though they are no longer surrounded by a membrane. The granules eventually disperse to fill the PVS (Fig. 3B), and the thickness of the cortical granule envelope can be quite substantial (Fig. 3B,C). In the

![Fig. 3. Transmission electron micrographs showing the cortical granule envelope in the PVS of fertilized human (A), mouse (B), and opossum oocytes (C). In the human, cortical granules have been exocytosed and are in the process of dispersing (arrows). In the mouse, the granules have already dispersed and formed the envelope (arrows). In the opossum, the envelope (*) is fully formed beyond the tips of the microvilli. ZP = zona pellucida.](image)
marsupial for example, it is as thick as the zona itself (Fig. 3C). The cortical granule envelope remains in the PVS throughout preimplantation development and is lost only after blastocysts hatch from the zona pellucida (Talbot and DiCarlantonio, 1984a).

COMPOSITION OF THE CORTICAL GRANULE ENVELOPE

The cortical granule envelope has not yet been isolated from mammalian oocytes and analyzed biochemically. However, it has been shown that the envelope is stabilized by ruthenium red, a polycation (Dandekar et al., 1992, 1995; Dandekar and Talbot, 1992), suggesting that the envelope is negatively charged probably due to glycosylation. Its charge might preclude diffusion of the envelope molecules through the zona, thereby retaining it in the PVS throughout preimplantation development. The idea that the envelope is enriched in carbohydrate is supported by the observation that lectins such as ConA and LCA bind to the envelope of fertilized hamster oocytes (Hoodbhoy and Talbot, 2001). Since the same lectins bind the cortical granules of unfertilized oocytes but do not bind the PVS of unfertilized oocytes recovered from the oviduct, it is reasonable to conclude that the envelope originates from glycosylated molecules released from the cortical granules during the cortical reaction. An antibody, ABL2, that recognizes the cortical granules of unfertilized mouse and hamster oocytes also binds to material in the PVS and on the oolemma of fertilized hamster oocytes (Fig. 4A,B) (Hoodbhoy et al., 2001). Binding of ABL2 to PVS material and to the surface of dividing blastomeres continues until the time of blastocyst hatching. ABL2 recognizes two bands of 56 and 62 kDa on Western blots of unfertilized oocytes. These bands are decreased in amount in zona free fertilized oocytes but equal in amount to unfertilized oocytes when fertilized oocytes are examined zona intact. ABL2 also labels cortical granules of rat, pig and bovine oocytes and it reacts with bands at 56 and 62 kDa on Western blots of these species. The relationship between these two proteins is not yet known, but the immunocytochemical and biochemical observations with the ABL2 antibody are consistent with p62/p56 being components of the cortical granule envelope. To date, these are the only proteins that have been identified as putative cortical granule envelope components. Because some ABL2 antibody binds to the oolemma after fertilization, it is possible that one protein is present in the envelope while the other is located on or in the oolemma. In mice, new synthesis of ABL2 binding antigens has been reported after fertilization beginning at about the two-cell stage with greatest synthesis at the eight-cell stage (Johnson and Calarco, 1980; Polak-Charcon et al., 1985). Likewise in hamsters, greater labeling was seen in the PVS space and on the blastomere surface of eight-cell embryos than at the one-cell stage (Hoodbhoy et al., 2001). These observations suggest that the cortical granule envelope material recognized by the ABL2 antibody is released and assembled at fertilization but that it is replenished and its amount is augmented during preimplantation development. p62/p56 have not yet been identified biochemically; however, interestingly, antibodies to sea urchin hyalin also react with the cortical granules of unfertilized hamster oocytes and with the cortical granule envelope of fertilized oocytes and preimplantation embryos (Hoodbhoy et al., 2000). On Western blots, the anti-hyalin antibody also recognizes p62/p56. Hyalin is a complex protein that is released into the PVS of sea urchin oocytes at fertilization, and plays a role in normal development and morphogenesis of sea urchins (Adelson and Humphreys, 1988; Pink and McClay, 1985; McClay and Pink, 1982). Mechanical removal of hyalin from sea urchin oocytes retards their growth. However, urchins can synthesize new hyalin that, when released, enables development to continue (Citkowitz, 1971). The ABL2 antigens p62/p58 are much smaller than sea urchin hyalin, but the immunological data suggest that hyalin and the ABL2 antigens share at least one epitope. There is not currently evidence that the ABL2 antigens block polyspermy. When injected in vivo into the oviduct at various times before fertilization, the ABL2 antibody did not increase the incidence of polyspermy (Hoodbhoy et al., 2001). However, this could be a null, not negative, result as we do not know that the ABL2 antibodies are function blocking with respect to polyspermy, and it is possible that other cortical granule components blocked polyspermy even though the
ABL2 antigens were inactivated by antibodies. Further work will be required to determine if p62/56 do play a role in blocking polyspermy.

ASSEMBLY OF THE CORTICAL GRANULE ENVELOPE

Little is known about the assembly of the cortical granule envelope. When cortical granules undergo exocytosis, their contents are released into the PVS by exocytosis (Dandekar et al., 1992; Dandekar and Talbot, 1992; Szollosi, 1967). The contents are easily identifiable as they remain transiently aggregated and form an electron dense clump about the size of the cortical granules inside oocytes. However, the granules in the PVS do appear to be slightly expanded, apparently due to partial dispersion of their contents. Although assembly has not been studied in detail beyond ultrastructural observation, the clumped material appears to eventually disperse to form an envelope that completely surrounds the fertilized oocyte (Dandekar et al., 1992, 1995; Dandekar and Talbot, 1992). The granules of the undispersed contents and the dispersed contents look similar. In an interesting study, Cran and Cheng (1986) observed that the dispersion of cortical granules in the PVS of pig oocytes following ionophore-induced exocytosis was related to the amount of calcium in the culture medium. At 1.8 mM calcium, exocytosis failed to occur. At 4.72 mM calcium, cortical granules were released but did not disperse, and at 7.64 mM calcium, released granules dispersed into the PVS. Thus, calcium appears to be important in dispersion of the cortical granule material, which would be the initial step in enabling the cortical granule envelope to assemble.

DOES THE CORTICAL GRANULE ENVELOPE BLOCK POLYSPERMY?

There are several mechanisms by which the cortical granule envelope could block polyspermy. After fertilization, the PVS enlarges significantly with the formation of the cortical granule envelope (Talbot and DiCarlantonio, 1984a). In unfertilized oocytes, the PVS is small, and sperm are likely to come in contact with the oolemma upon entering the PVS. The enlarged PVS of the fertilized oocyte could pose an orientation problem for sperm after penetrating the zona. When the PVS is large, it may be more difficult for the side of the sperm head, where the fusogenic portion of the plasma membrane resides, to engage and bind to the microvilli of the oolemma. Thus bringing the gametes into proper alignment for fusion may be less likely to occur in fertilized oocytes with a large PVS than in unfertilized oocytes with a smaller PVS where the sperm head would be more likely to encounter the oolemma.

It is well established that some components in mammalian cortical granules can enter the zona pellucida and modify it to prevent polyspermy (Barros and Yanagimachi, 1971, 1972; Gwatkin et al., 1973; Wolf and Hamada, 1977). While the zona reaction clearly occurs as the oocytes were aged. These data with human oocytes subjected to SUZI would be consistent with the idea that the cortical granule envelope was at least partially formed in aged oocytes and prevented sperm fusion with the oolemma. Removal of the
zona results in dispersion of the cortical granule envelope (Talbot and DiCarlantonio, 1984a), which could explain why zona-free oocytes readily permit sperm fusion (Soupart and Strong, 1975; Tesarik, 1989). It is also important to note that the oolemma of hamsters does not lose its ability to fuse with sperm until the 8-cell stage (Usui and Yanagimachi, 1976; Zuccotti et al., 1991), suggesting that if a block to polyspermy exists in hamsters after passage through the zona, the block would be in the PVS.

Unlike many other mammals, rabbits do not appear to have a strong block to polyspermy at the zona level (Austin, 1961; Braden et al., 1954). Numerous sperm can enter the PVS during fertilization yet oocytes remain monospermic. This suggests that rabbits have a powerful block to polyspermy at the level of the oolemma and indeed the charge density of the rabbit oolemma, as assessed by labeling with charged ferric colloid particles, increases after fertilization (Cooper and Bedford, 1971). However, a block to polyspermy in rabbits could likewise indicate a strong block at the level of the cortical granule envelope. In an interesting study on rabbits, McCulloh et al. (1987) found that zona enclosed oocytes were better able to prevent polyspermy than zona-free oocytes, even though there is no block to polyspermy at the level of the zona in rabbits. This could be due to the presence of the cortical granule envelope in the PVS of zona intact oocytes. This envelope would be lost after zona removal thereby making the oocytes more vulnerable to polyspermy. It would be interesting to compare the envelope of rabbits and other mammals to determine if a block to polyspermy exists at this level.

Further experimental evidence for the existence of a PVS block to polyspermy has come from a re-insemination study using mice (Maluchnik and Borsuk, 1994). Fertilized oocytes freed of the zona before extrusion of the second polar body were capable of fusing with sperm. However, these same oocytes had not been able to bind motile sperm in the PVS prior to zona removal. These data support the idea that a block to polyspermy exists at the level of the PVS. Supplementary motile sperm do not bind to the oolemma; however, sperm can fuse with the oolemma if the zona and the cortical granule envelope are removed. While these data identify a block to polyspermy at the level of the PVS, they do not identify what in the PVS does the blocking. The major component of the PVS after fertilization is the cortical granule envelope, which would have been removed in the re-insemination experiments. These data support the idea that the cortical granule envelope does play a role in blocking polyspermy by preventing supplementary sperm from binding to the oolemma.

While the cortical granule envelope appears to play a role in blocking polyspermy when more than one sperm gains access to the PVS, it is not yet known if p62/p56 actually perform this function or if other envelope components block polyspermy. In vivo experiments in which the ABL2 antibody or an antibody to sea urchin hyaluronan were injected into the ampulla of hamster oviducts prior to fertilization did not result in an increased incidence of polyspermy (Hoodbhoy et al., 2000, 2001). However, this could be a null result as it is possible that p62/p56 were neutralized by the antibody but backed up by another protein(s) in the cortical granule envelope, or it is possible that these antibodies were not capable of blocking this putative function of p62/p56, or that other proteins in the cortical granule envelope block polyspermy. Additional studies will need to be done to establish if p62/p56 do have a role in blocking polyspermy.

DOES SMOKING AFFECT BLOCKS TO POLYSPERMY

It is well established that smokers have an increased incidence of infertility and that there may be multiple mechanisms that account for this (Phipps et al., 1987; Stillman et al., 1986). Moreover, numerous studies done in human in vitro fertilization labs generally confirm that smoking is detrimental to reproductive processes (Klonoff-Cohen et al., 2001). Cigarette smoke contains over 4,000 chemicals (EPA, 1992), many of which are recognized toxicants and some of which are only now being identified as potentially dangerous (Ji et al., 2002). It is probable that many of the toxicants in cigarette smoke gain access to oocytes. Cotinine, a major metabolite of nicotine, has been reported to be detectable at high levels in the follicular fluid of both active and passive smokers (Zenzes et al., 1996). Nicotine has been reported to increase the incidence of polyspermy in sea urchin oocytes (Longo and Anderson, 1970; Rothschild, 1954). The mechanism for this action is not yet established; however, sea urchin oocytes do have nicotinic acetylcholine receptors in their plasma membranes (Ivonneit and Chambers, 1997), suggesting nicotine could act directly on the plasma membrane to affect polyspermy. In a structural study of sea urchin eggs undergoing fertilization while exposed to nicotine, it was observed that the cortical reaction and formation of the activation calyx occurs more quickly in nicotine treated oocytes than in controls (Longo and Anderson, 1970). It is not clear if this effect was due to multiple sperm fusing with the oocytes thereby initiating the reaction at several places or if the acceleration of the cortical reaction and calyx formation is a direct consequence of nicotine. The effects of nicotine on polyspermy in mammalian oocytes are not well characterized. However, since cotinine, a major metabolite of nicotine, has been reported to accumulate in follicular fluid (Zenzes et al., 1996) and could thereby gain access to the PVS, the possibility that chemicals in cigarette smoke affect polyspermy should be investigated further. It would be interesting, for example, to determine if smokers undergoing in vitro fertilization have a higher incidence of polyspermy than non-smokers.

SUMMARY AND CONCLUSIONS

The PVS of mammalian oocytes is a dynamic zone that undergoes changes in size and composition during development. An extracellular matrix first appears in the PVS of follicular oocytes after the LH surge, and later the PVS is modified by the addition of oviduct secretory glycoprotein. The effects of the matrix prior to fertilization have not been investigated but could affect fertilization, as hyaluronan, a molecule that can inhibit fusion, is present in the PVS of unfertilized oocytes. Following fertilization, a new matrix, the cortical granule envelope, appears in the PVS. In oocytes
where the cortical granule contents do not completely disperse to form this matrix, polyspermy levels are increased. This could be because granule contents involved in the zona reaction are not dispersed and hence are not able to enter the zona and/or because the cortical granule envelope does not form properly, and supplementary sperm entering the PVS are not blocked from binding to the oolemma. Most work on polyspermy blocks have focused on the zona pellucida and on the plasma membrane of the oocyte. Often in studies of the plasma membrane block, zona free oocytes are used so that the cortical granule envelope would not be present. New work is needed to examine the functions of the cortical granule envelope directly and to determine if it plays a role in blocking polyspermy. Clearly the PVS is an interesting and complex compartment surrounding mammalian oocytes. Much work remains to be done to define the matrices present in this space, how some components are selectively retained in the space while other pass freely away from the space through the zona, and what roles the matrix in the PVS plays in fertilization and preimplantation development.

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POLYSPERMY AND THE PVS


