Importance of Sperm Capacitation, Removal of Cumulus Matrix, Acrosome Reaction, and Sperm-egg Fusion in the Process of *In Vitro* Fertilization

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ABSTRACT

Mammalian fertilization is a complex cascade process consisting of sperm migration through the female reproductive tract, physiological changes to sperm such as sperm capacitation and acrosome reaction, and sperm-egg interaction in the oviduct *in vivo*. On the other hand, *in vitro* fertilization (IVF) is a process by which egg cells are fertilized by sperm outside the body: *in vitro*. IVF has been used for a variety of purposes in reproductive biotechnology for human and animals. The discovery of sperm capacitation in 1951 promoted the development of IVF technology. In the initial stage of IVF, sperm capacitation in preincubation medium was shown to be essential to fuse with eggs. Besides, sperms should detour some of the *in vivo* regulations for IVF. This review introduces a general mammalian fertilization process, including sperm capacitation, removal of cumulus matrix, acrosome reaction, and sperm-egg fusion and focuses on the roles of key biochemical molecules, signal mechanisms, and genes involved during IVF and novel results of sperm-oocyte interaction elucidated in various gene-knockout mice models.

(Key words : IVF, capacitation, acrosome reaction, sperm-egg fusion)

INTRODUCTION

While invertebrates whose motile sperm is immediately able to fertilize eggs reproduce by external fertilization, most mammals employ internal fertilization via multiple sophisticated processes. In the initial stage of fertilization, a mammalian sperm should be capacitated to fertilize an egg, which is the physiological changes that confer on the sperm the ability to fertilize, collectively called "capacitation" (Eliasson, 1966; Ikawa *et al*., 2010). Sperm capacitation happens in the female reproductive tract. When a sperm starts to move for fertilization through the ejaculation, a sperm is in a decapacitated state and has low motility because cholesterol is rich in the sperm plasma membrane. Therefore, the excessive cholesterol should be removed for sperm capacitation. Several factors such as methyl-β-cyclodextrin (MBCD) and albumin are known to be involved with the removal of cholesterol in the plasma membrane (Choi and Toyoda 1998; Ilangumaran and Hoessli, 1998; van Gestel *et al*., 2005; Kato *et al*., 2010).

The capacitated sperms pass through the cumulus matrix, which is surrounding an oocyte and beneficial for fertilization *in vivo* (Rodger and Bedford, 1982; Tanghe *et al*., 2002), using several surface molecules and then bind to the zona pellucida (ZP) after dispersing the cumulus cells. Next, acrosomal exocytosis so called acrosome reaction (AR) is required for the completion of next sequential processes of fertilization, including ZP binding, penetration of ZP, and sperm-egg fusion (Gupta and Bhandari, 2011).

In this review we discuss on the mechanisms underlying mammalian *in vitro* fertilization (IVF) which has been studied recently. IVF is a process by which egg cells are fertilized by sperms outside the body: *in vitro*. IVF is a major treatment in infertility when other methods of assisted reproductive technology have failed. Therefore, IVF has become an important application in human infertility clinics and animal reproduction during the last several decades. For IVF, sperms bypass some of the *in vivo* regulations. Here, we focus on the studies regarding sperm capacitation, removal of cumulus cells, AR, ZP binding, and egg-sperm fusion in the IVF process.

† This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government (no. 2011-0015385) to KCC and the KRIBB initiative program to KHN.

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1. Sperm Capacitation

In order to be capacitated, cholesterol in sperm plasma membrane should be removed by an influx of Ca^{2+} ions and increase in internal-acrosomal pH, which result in phospholipid redistribution within the sperm plasma membrane and the activation of sperm adenyl cyclase. Changes in plasma membrane phospholipid content and distribution (Snider and Clegg, 1975) are followed by cholesterol efflux during capacitation that dramatically reduces the ratio of cholesterol to phospholipid in sperm plasma membrane (Davis, 1981).

And reproductive tract fluids such as the oviductal and follicular fluids also act to remove cholesterol by cholesterol binding proteins during capacitation *in vivo* (Langlais *et al*., 1988). After passing through the uterus, a sperm moves to the oviduct where cumulus-oocyte complexes (COCs) secrete the progesterone that is known as the sperm chemoattractant in humans and rabbits (Guidobaldi *et al*., 2008; Oren-Benaroya *et al*., 2008). A sperm is hyperactivated in the last stage of capacitation and shows the increase in amplitude, asymmetrical beating, frequency of the flagellar movement, and lateral displacement of the sperm head, altogether resulting in an increased sperm velocity (Suarez 1996; Olson *et al*., 2011), which is calcium dependent (Yanagimachi and Usui, 1974; Demott and Suarez, 1992; Holtzmann *et al*., 2011). Hyperactivated sperms are detached from the oviductal reservoir. The gradual release of sperm from the isthmus also helps to reduce the number of sperm available at the point of fertilization and can avoid polyspermy (Hunter, 1996). A study of the time relationship of fertilization in the mice indicated that the capacitation occurs within a very short time (about 1 hour) (Braden and Austin, 1954).

However, IVF adopts other regulation processes in sperm capacitation. IVF unlike *in vivo* fertilization must remove cholesterol by artificial means (Langlais *et al*., 1988). The major material for cholesterol removal is MBCD that promotes sperm capacitation *in vitro* by decreasing the amount of cholesterol in the plasma membrane. The concentration of MBCD is crucial for effective capacitation induction (Choi and Toyoda, 1998; Ilangumaran and Hoessli, 1998; van Gestel *et al*., 2005; Kato *et al*., 2010) and also related with exposure of some proteins that are involved in capacitation-dependent processes and ZP binding (van Gestel *et al*., 2005). The physiological meaning of raft redistributing in the sperm surface is to create protein complexes involved in ZP binding (Tanphaichitr *et al*., 2007; van Gestel *et al*., 2007). Using a high concentration of albumin in culture medium is also essential to decrease the sperm cholesterol/phospholipid ratio (Davis *et al*., 1980; Harrison and Gadella, 2005; Travert *et al*., 2009). Albumin also reduces the amount of sialic acid, ganglioside, and triglyceride in the sperm plasma membrane (Harrison and Gadella, 2005). Serum albumin or heparin is commonly used for *in vitro* capacitation for this purpose. Sodium bicarbonate is also required in *in vitro* capacitation media (Valencia *et al*., 1984; Jaiswal *et al*., 1998; Dapino *et al*., 2009). The changes in sperm plasma membrane and possibly in the outer acrosomal membrane result in increased fluidity and fuseability of sperm membrane (Gadella *et al*., 1999). In addition, IVF usually requires thousands of sperm hyperactivated in sperm capacitation to increase the rate of success.

2. The Removal of Cumulus Matrix

After sperm capacitation, a sperm interacts with and penetrates the barrier of cumulus matrix that surrounds the oocyte and is rich in proteins and carbohydrates such as hyaluronan, an unsulfated glycosaminoglycan. Hyaluronic acid (also known as hyaluronan) is composed of repeating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid and is one of the most common glycosaminoglycans which are present in the extracellular matrix of connective tissues (Kreil, 1995; Baba *et al*., 2002; Hong *et al*., 2009). The mammalian COCs are ovulated into the peritoneal or bursal cavity, picked up by the oviductal infundibulum, and transported into the ampulla where fertilization takes place (Talbot *et al*., 1999). The COCs that are dispersed by sperm hyaluronidase are unique to the egg of mammals (Bedford and Kim, 1993). However, that cumulus removal affects fertilization rates is species dependent (Vergara *et al*., 1997). Though the removal of the cumulus matrix by hyaluronidase increased *in vitro* fertilization rates in human (Lavy *et al*., 1988; Vergara *et al*., 1997), the removal in cattle or pigs decreased the sperm penetration (Wang *et al*., 1995; Zhang *et al*., 1995; Suzuki *et al*., 2000; Fatehi *et al*., 2002). Nevertheless, the cumulus matrix is beneficial for fertilization. Genetic deletion of genes involved in synthesizing and stabilizing the COCs suppresses fertilization *in vivo* (Rodger and Bedford 1982; Tanghe *et al*., 2002). The cumulus cells in the COCs also supply the oocytes with nutrients; pre-ovulatory oocytes are at a relatively long distance from the vascular supply of oxygen, nutrients, and signals, and under meiotic arrest (Dekel, 1988; Eppig, 1989; Tanghe *et al*., 2002).

In order to pass through the COCs, a sperm secretes the hya-

luronidase enzymes such as PH20/SPAM1 and HYAL5 that are present on the sperm surface probably to support sperm penetration through cumulus matrix and dispersion of cumulus cells during fertilization (Myles and Primakoff 1997; Kim *et al*., 2005). Capacitation appears to be prerequisite for successful cumulus penetration (Rogers and Morton, 1973), because proteolytic processing, unmasking, or release of the sperm hyaluronidases occurs at that time. In contrast, premature acrosomal exocytosis disturbs cumulus passage of sperms (Tesarik, 1989). Even under the presence of apigenin (also known as hyaluronidase inhibitor), however, a sperm could penetrate the cumulus cells and bind with ZP. Therefore, sperm hyaluronidase may just assist a sperm in accessing to ZP not by the hyaluronan-degrading activity but by unknown function(s) (Kang *et al*., 2010). Sperm adhesion molecule 1 (SPAM1), which was the first identified as ZP-binding protein PH-20 (Primakoff *et al*., 1985), is present on the plasma membrane of both acrosome-intact and acrosome-reacted sperm (Baba *et al*., 2002). However, SPAM1 was later proven to have hyaluronidase activity and to be involved in passage of sperm through the COCs (Primakoff *et al*., 1988; Lin *et al*., 1994). The originally described ZP-binding ability of SPAM1 is not prerequisite because sperms of *Spam1*-knockout mice have been shown to bind with the ZP as well as be fertile, although the mutant sperm shows a decreased ability to disperse cumulus cells *in vitro* (Baba *et al*., 2002). *Spam1*-defective sperms preserve about 40% of their hyaluronidase activity. Although hyaluronoglucosaminidase 5 (Hyal5) was demonstrated to be an additional sperm specific hyaluronidase (Kim *et al*., 2005), the fertility of sperms from *Hyal5*-knockout mice was analogous to that of wild-type mice, both *in vitro* and *in vivo* (Kimura *et al*., 2009). Therefore, it looks like that Spam1 has more important roles for sperm to penetrate the cumulus cells than Hyal5 (Kim *et al*., 2008).

3. Acrosome Reaction (AR) and Penetration to Zona Pellucida (ZP)

The tip of the sperm head is covered with a golgi-derived exocytic organelle, an acrosome. The capacitating process of a sperm is accompanied by acrosome exocytosis called AR, which is essential for a sperm to fuse with an egg and a prerequisite for ZP binding (Saling and Storey, 1979; Cross and Meizel, 1989; Lai *et al*., 1996). AR also can be induced *in vitro* with solubilized ZP (Florman and Storey, 1982; Cherr *et al*., 1986; Fukami *et al*., 2001). However, the intact ZP is not enough to induce AR (Baibakov *et al*., 2007). Human cumulus cells secret progesterone to induce AR (Patrat *et al*., 2000). Moreover, 20～40% of capacitated mice sperms spontaneously undergo AR in IVF media such as TYH and HTF and those sperms are efficiently able to fertilize eggs *in vitro* without the cumulus cells or solubilized ZP (Nakagata *et al*., 1997; Vergara *et al*., 1997). The mechanism of the AR has been well known. Briefly, a transient calcium influx gives rise to the activation of phospholipase C (PLC), which incurs inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol 4,5 bisphosphate (PIP2). IP3 releases calcium from intracellular stores, and DAG mediates PKC activation and phosphorylation of substrate proteins. These early events promote a following calcium influx by way of transient receptor potential cation channels (TRPCs), which lead to the complete AR (Fukami *et al*., 2001; Buffone *et al*., 2009; Ikawa *et al*., 2010). The soluble NSF attachment protein receptor (SNARE) complex controls the sperm AR (Kierszenbaum 2000). Sperms from hydrocephaly with hop gait (Hyh) mice, which have a spontaneous point mutation in the α-SNAP-encoding gene (*Napa*), exhibit severely impaired fertility (Batiz *et al*., 2009). Mice lacking complexin-1 (*Cplx1*), which associates with the SNARE complex and modulates its function (Reim *et al*., 2001; Maximov *et al*., 2009), also generate sperms with an impaired ability to undergo progesterone-induced ARs. These genes are related with the SNARE complex. Although *Napa* and *Cplx1* disrupted mutant sperms are severely damaged in their ability to undergo ligand-induced ARs, these sperms are still able to fertilize with eggs *in vitro* (Roggero *et al*., 2007; Zhao *et al*., 2007; Maximov *et al*., 2009).

After dispersing the cumulus cells and passing through the COCs, sperm reaches the ZP, the last obstacle before meeting the egg, undergoing AR. The main elements of the ZP in mice are three glycoproteins, ZP1, ZP2 and ZP3. Whereas, in humans, it is composed of four components (ZP1, ZP2, ZP3 and ZP4) (Gupta and Bhandari, 2011). In mice, O-linked glycans of ZP3 function as the primary sperm receptor and are involved in the AR, whereas in humans, N-linked glycans of ZP1, ZP3, and ZP4 are critical for AR induction. ZP4 also has been identified in some species, but its species-specific function remains to be determined (Bleil and Wassarman, 1980; 1983; Arnoult *et al*., 1996; Lefievre *et al*., 2004; Gupta and Bhandari, 2011). During the passage of a sperm through the ZP, ZP2 is known to function as a secondary receptor for acrosome-reacted sperm. If an oocyte has been fertilized with one sperm, ZP2 is converted to ZP2f by an oocyte secretory enzyme(s) to prevent polyspermy (Bleil *et al*., 1988; Kurasawa *et al*., 1989). ZP1 is

known as cross-linked ZP2/ZP3 heterodimers and makes the filamentous structure of the ZP (Green, 1997). Eggs can form ZP in the absence of either ZP1 or ZP2 and sperms can fertilize these eggs (Rankin *et al*., 1999; Rankin *et al*., 2001). However, *Zp3*-knockout mice could not form ZP (Liu *et al*., 1996; Rankin *et al*., 1996). ZP not only functions as a receptor for sperm but also acts as a species-specific barrier (Ikawa *et al*., 2010).

Five knockout mice strains have now been reported to show defective sperm-ZP binding (*Clgn*-, *Ace*-, *Adam1a*-, *Adam2*-, and *Adam3*-knockout mice) and these genes are also related with sperm motility in the mice (Yamaguchi *et al*., 2009). The disruption of calmegin (*Clgn*) impairs Adam1a/Adam2 and Adam1b/Adam2 heterodimerization, and because a disintegrin and metalloprotease (Adam) family members control the quantity of Adam3 on the sperm surface, this induces the absolute loss of Adam2 and Adam3 from the surface of mature sperm (Ikawa *et al*., 2001; Yamaguchi *et al*., 2006; Ikawa *et al*., 2010). In *Adam1b*-knockout mice, Adam2 was also found to disappear from the sperm, but Adam3 remained intact and the sperm were fertile (Kim *et al*., 2006). The Adam3 protein can shortly bind to ZP (Kim *et al*., 2005). Adam3 requires the joint action of Clgn and Adam1a/Adam2 for its quality control and maturation during spermatogenesis. The mechanism of infertility caused by *Ace* disruption has been remained unclear for many years, since gross levels of ADAMs in sperm are not influenced by Ace. However, a substantial decrease in the amount of Adam3 in membrane microdomains or an erroneous distribution of ADAM3 in *Ace*-knockout mice might cause the defective ZP-binding phenotype (Fukami *et al*., 2001; Yamaguchi *et al*., 2006; Buffone *et al*., 2009; Ikawa *et al*., 2010). Although Adam3 is the most important factor in the sperm-ZP binding, other proteins including the 56-kDa ZP3 receptor (ZP3R) and zonadhesin (ZAN) are involved in human sperm binding to ZP. ZP3R is a peripheral membrane glycoprotein that has been suggested to be a receptor for ZP3 in the mice (Cheng *et al*., 1994; Bookbinder *et al*., 1995). However, the receptor is not essential for mice fertilization (Muro *et al*., 2011). ZAN is a large sperm head protein with multiple isoforms. ZAN is known to mediate a species-specific ZP adhesion and *Zan*-knockout male mice are fertile because their spermatozoa retain an adhesion capability (Gao and Garbers, 1998; Tardif *et al*., 2010).

Proteolytic cleavage of ZP proteins by sperm surface proteases clears a road for the incoming sperm. Acrosin (Acr) is an acrosomal enzyme with chymotryptic activity that is released during AR (Kawano *et al*., 2010). Despite several papers supporting an important role for Acr in ZP binding and penetration, sperms from *Acr*-knockout mice can still fertilize eggs, nevertheless with a slight delay compared with wild-type sperm (Baba *et al*., 1994; Adham *et al*., 1997). Five more testis-specific serine proteases (Tesp) have been identified and named as Tesp1, 2, 3, 4 and 5 (Kohno *et al*., 1998; Ohmura *et al*., 1999; Honda *et al*., 2002; Kondoh *et al*., 2005). *Tesp5*-deficient epididymal sperms are severely defective in the ability to undergo the ZP-induced AR, to penetrate ZP, and to fuse with the egg membrane *in vitro*. Thus, Tesp5 may play an important role in AR and penetration to ZP (Kim *et al*., 2008).

4. Sperm-egg Fusion

After passing through the barrier of ZP, a sperm fuses with the egg plasma membrane (Bedford, 1968; Stein *et al*., 2004; Inoue and Okabe, 2008). Only acrosome-reacted sperms can fuse with eggs. Sperm fusogens, which are located inner-acrosome membrane in a fresh sperm, are exposed after AR. One of the fusogens, IZUMO, named after Japanese Shinto shrine, is an immunoglobulin superfamily protein and essential for sperm-egg fusion. Izumo1 that is not exposed before AR relocates from the anterior head of a sperm to the region in which fusion will happen (Gupta and Bhandari, 2011). Male mice lacking *Izumo1* are entirely infertile with no successful fusion with eggs, though the sperm from the mice can penetrate ZP (Inoue *et al*., 2005). However, sperms of infertile men have the intact IZUMO. For that reason, IZUMO is not the major cause of male infertility in human (Hayasaka *et al*., 2007). Testis-specific serine kinase 6 (Tssk6) is a male germ cell-specific serine kinase. The sperms from mice lacking *Tssk6* have defects in ZP binding and cannot redistribute Izumo1 properly (Spiridonov *et al*., 2005; Sosnik *et al*., 2009). The cysteine-rich secretory proteins (CRISP) (Cohen *et al*., 2007), including epididymal protein DE (CRISP1) and testicular protein TXP1 (CRISP2), also have been involved in sperm-egg fusion (Busso *et al*., 2007; Busso *et al*., 2007; Cohen *et al*., 2008). Both male and female mice immunized with recombinant CRISP1 displayed reduced fertility (Ellerman *et al*., 2008).

In female eggs, CD9 and CD81 on the plasma membrane belong to the tetraspanin superfamily and form a complex with $\alpha 3 \beta 1$ (Kaji *et al*., 2000; Miyado *et al*., 2000; Tanigawa *et al*., 2008). CD9 is ubiquitously expressed and expected to function in various cells. *Cd9*-deficient mice are infertile. Although

Table 1. The major components of fertilization

Sperm capacitation (Hyperactivation)
Commonly related with sperm motility: Clgn, Ace, Adam1a, Adam2, or Adam3, Catsper1
In vivo: Oviductal and follicular fluids
In vitro: MBCD, Heparin, BSA, Ca^{2+} influx
Cumulus removal in cumulus-oocyte complexes
Oocyte: Hyaluronic acid in cumulus matrix
Sperm: Hyaluronidases (SPAM1/PH20, HYAL5)
Acrosome reaction
Oocyte: Zp1, ZP2, ZP3, ZP4, T-SNARE, progesterone, solubilized ZP
Sperm: V-SNARE (Plcd4, Hyh, Cplx1), Clgn, Ace, Adam1a, Adam2, or Adam3, ZP3r, ZAN, Acr, Tesp5, proteasome
Sperm-egg fusion
Oocyte: CD9, CD81
Sperm: IZUMO1, TSSK6, CRISP1, CRISP2

* MBCD; Methyl beta cyclodextrin, BSA; Bovine serum albumin, ZAN; Zonadhesin, Acr; Acrosin.

they normally produce mature oocytes, these oocytes are not able to fuse with sperms (Kaji *et al*., 2000; Miyado *et al*., 2000). CD81, which has a similar structure and function to CD9, is also involved in sperm fusion. However, the effects of *Cd81*-knockouts are less drastic than those of *Cd9*-knockouts (Rubinstein *et al*., 2006; Tanigawa *et al*., 2008). Although both CD9 and CD81 are present on the surface of murine oocytes, the expression level of CD9 is not affected by a deficiency of CD81 and vice versa. CD9 is expressed on the oocyte microvilli (Runge *et al*., 2007), whereas CD81 is located in microdomain-like structures between microvilli. However, both CD9 and CD81 are concentrated at the sperm binding site. The *Cd9-Cd81* double knockout mice are completely infertile (Rubinstein *et al*., 2006). Therefore, CD9 and CD81 are important for coordinating the sperm-egg fusion process in mice. The direct interaction between sperm lacking *Izumo1* and egg lacking *Cd9* has not been studied yet.

CONCLUSION

In this review, we highlighted diverse factors involved in IVF process in animals. During the last several decades, IVF has become an important application in human infertility clinics and animal reproduction. The sperm capacitation is essential for the successful IVF performance, which is the starting process of a complex mammalian fertilization cascade followed by cumulus removal, AR, ZP binding, and sperm-egg fusion. The more clear understanding on the mechanism of sperm-oocyte interaction during fertilization *in vivo* and *in vitro* will be much helpful for infertility treatments in human and animals. The major components in IVF process are shown in Table 1. IVF is also a powerful tool of mass production for experimental animals including genetically engineered mice. More detailed mechanism(s) of sperm capacitation, cumulus removal, AR, ZP binding, and sperm-egg fusion should be uncovered, which will be applied to enhance a higher IVF rate in animals and human.

REFERENCES

Adham IM, Nayernia K and Engel W. 1997. Spermatozoa lacking acrosin protein show delayed fertilization. Mol. Reprod. Dev. 46:370-376.

Arnoult C, Zeng Y and Florman HM. 1996. ZP3-dependent activation of sperm cation channels regulates acrosomal secretion during mammalian fertilization. J. Cell. Biol. 134: 637-645.

Baba D, Kashiwabara S, Honda A, Yamagata K, Wu Q, Ikawa

M, Okabe M and Baba T. 2002. Mouse sperm lacking cell surface hyaluronidase PH-20 can pass through the layer of cumulus cells and fertilize the egg. J. Biol. Chem. 277: 30310-30314.

Baba T, Azuma S, Kashiwabara S and Toyoda Y. 1994. Sperm from mice carrying a targeted mutation of the acrosin gene can penetrate the oocyte zona pellucida and effect fertilization. J. Biol. Chem. 269:31845-31849.

Baibakov B, Gauthier L, Talbot P, Rankin TL and Dean J. 2007. Sperm binding to the zona pellucida is not sufficient to induce acrosome exocytosis. Development 134:933-943.

Batiz LF, De Blas GA, Michaut MA, Ramirez AR, Rodriguez F, Ratto MH, Oliver C, Tomes CN, Rodriguez EM and Mayorga LS. 2009. Sperm from hyh mice carrying a point mutation in alphaSNAP have a defect in acrosome reaction. PLoS One 4:e4963.

Bedford JM and Kim HH. 1993. Cumulus oophorus as a sperm sequestering device, *in vivo*. J. Exp. Zool. 265:321-328.

Bedford JM. 1968. Ultrastructural changes in the sperm head during fertilization in the rabbit. Am. J. Anat. 123:329-358.

Bleil JD and Wassarman PM. 1980. Mammalian sperm-egg interaction: Identification of a glycoprotein in mouse egg zonae pellucidae possessing receptor activity for sperm. Cell 20:873-882.

Bleil JD and Wassarman PM. 1983. Sperm-egg interactions in the mouse: Sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. Dev. Biol. 95:317-324.

Bleil JD, Greve JM and Wassarman PM. 1988. Identification of a secondary sperm receptor in the mouse egg zona pellucida: Role in maintenance of binding of acrosome-reacted sperm to eggs. Dev. Biol. 128:376-385.

Bookbinder LH, Cheng A and Bleil JD. 1995. Tissue- and species-specific expression of sp56, a mouse sperm fertilization protein. Science 269:86-89.

Braden AW and Austin CR. 1954. Fertilization of the mouse egg and the effect of delayed coitus and of hot-shock treatment. Aust. J. Biol. Sci. 7:552-565.

Buffone MG, Rodriguez-Miranda E, Storey BT and Gerton GL. 2009. Acrosomal exocytosis of mouse sperm progresses in a consistent direction in response to zona pellucida. J. Cell Physiol. 220:611-620.

Busso D, Cohen DJ, Maldera JA, Dematteis A and Cuasnicu PS. 2007. A novel function for CRISP1 in rodent fertilization: Involvement in sperm-zona pellucida interaction. Biol. Reprod. 77:848-854.

Busso D, Goldweic NM, Hayashi M, Kasahara M and Cuasnicu PS. 2007. Evidence for the involvement of testicular protein CRISP2 in mouse sperm-egg fusion. Biol. Reprod. 76:701-708.

Cheng A, Le T, Palacios M, Bookbinder LH, Wassarman PM, Suzuki F and Bleil JD. 1994. Sperm-egg recognition in the mouse: Characterization of sp56, a sperm protein having specific affinity for ZP3. J. Cell. Biol. 125:867-878.

Cherr GN, Lambert H, Meizel S and Katz DF. 1986. *In vitro* studies of the golden hamster sperm acrosome reaction: Completion on the zona pellucida and induction by homologous soluble zonae pellucidae. Dev. Biol. 114:119-131.

Choi YH and Toyoda Y. 1998. Cyclodextrin removes cholesterol from mouse sperm and induces capacitation in a protein-free medium. Biol. Reprod. 59:1328-1333.

Cohen DJ, Busso D, Da Ros V, Ellerman DA, Maldera JA, Goldweic N and Cuasnicu PS. 2008. Participation of cysteine-rich secretory proteins (CRISP) in mammalian sperm-egg interaction. Int. J. Dev. Biol. 52:737-742.

Cohen DJ, Da Ros VG, Busso D, Ellerman DA, Maldera JA, Goldweic N and Cuasnicu PS. 2007. Participation of epididymal cysteine-rich secretory proteins in sperm-egg fusion and their potential use for male fertility regulation. Asian J. Androl. 9:528-532.

Cross NL and Meizel S. 1989. Methods for evaluating the acrosomal status of mammalian sperm. Biol. Reprod. 41: 635-641.

Dapino DG, Teijeiro JM, Cabada MO and Marini PE. 2009. Dynamics of heparin-binding proteins on boar sperm. Anim. Reprod. Sci. 116:308-317.

Davis BK, Byrne R and Bedigian K. 1980. Studies on the mechanism of capacitation: Albumin-mediated changes in plasma membrane lipids during *in vitro* incubation of rat sperm cells. Proc. Natl. Acad. Sci. USA. 77:1546-1550.

Davis BK. 1981. Timing of fertilization in mammals: Sperm cholesterol/phospholipid ratio as a determinant of the capacitation interval. Proc. Natl. Acad. Sci. USA. 78: 7560-7564.

Dekel N. 1988. Regulation of oocyte maturation. The role of cAMP. Ann. N.Y. Acad. Sci. 541:211-216.

Demott RP and Suarez SS. 1992. Hyperactivated sperm progress in the mouse oviduct. Biol. Reprod. 46:779-785.

Eliasson R. 1966. Cholesterol in human semen. Biochem. J. 98:242-243.

Ellerman DA, Busso D, Maldera JA and Cuasnicu PS. 2008. Immunocontraceptive properties of recombinant sperm protein DE: Implications for the development of novel contraceptives. Fertil. Steril. 89:199-205.

Eppig JJ. 1989. The participation of cyclic adenosine monophosphate (cAMP) in the regulation of meiotic maturation of oocytes in the laboratory mouse. J. Reprod. Fertil. Suppl. 38:3-8.

Fatehi AN, Zeinstra EC, Kooij RV, Colenbrander B and Bevers MM. 2002. Effect of cumulus cell removal of *in vitro* matured bovine oocytes prior to *in vitro* fertilization on subsequent cleavage rate. Theriogenology 57:1347-1355.

Florman HM and Storey BT. 1982. Mouse gamete interactions: The zona pellucida is the site of the acrosome reaction leading to fertilization *in vitro*. Dev. Biol. 91:121-130.

Fukami K, Nakao K, Inoue T, Kataoka Y, Kurokawa M, Fissore RA, Nakamura K, Katsuki M, Mikoshiba K, Yoshida N and Takenawa T. 2001. Requirement of phospholipase Cdelta4 for the zona pellucida-induced acrosome reaction. Science 292:920-923.

Gadella BM, Flesch FM, van Golde LM and Colenbrander B. 1999. Dynamics in the membrane organization of the mammalian sperm cell and functionality in fertilization. Vet. Q. 21:142-146.

Gao Z and Garbers DL. 1998. Species diversity in the structure of zonadhesin, a sperm-specific membrane protein containing multiple cell adhesion molecule-like domains. J. Biol. Chem. 273:3415-3421.

Green DP. 1997. Three-dimensional structure of the zona pellucida. Rev. Reprod. 2:147-156.

Guidobaldi HA, Teves ME, Unates DR, Anastasia A and Giojalas LC. 2008. Progesterone from the cumulus cells is the sperm chemoattractant secreted by the rabbit oocyte cumulus complex. PLoS. One 3:e3040.

Gupta SK and Bhandari B. 2011. Acrosome reaction: Relevance of zona pellucida glycoproteins. Asian J. Androl. 13:97-105.

Harrison RA and Gadella BM. 2005. Bicarbonate-induced membrane processing in sperm capacitation. Theriogenology 63: 342-351.

Hayasaka S, Terada Y, Inoue N, Okabe M, Yaegashi N and Okamura K. 2007. Positive expression of the immunoglobulin superfamily protein IZUMO on human sperm of severely infertile male patients. Fertil. Steril. 88: 214-216.

Holtzmann I, Wolf JP and Ziyyat A. 2011. [Sperm reservoir in mice: Involvement of ADAMs]. Gynecol. Obstet. Fertil. 39:630-632.

Honda A, Yamagata K, Sugiura S, Watanabe K and Baba T. 2002. A mouse serine protease TESP5 is selectively included into lipid rafts of sperm membrane presumably as a glycosylphosphatidylinositol-anchored protein. J. Biol. Chem. 277:16976-16984.

Hong SJ, Chiu PC, Lee KF, Tse JY, Ho PC and Yeung WS. 2009. Cumulus cells and their extracellular matrix affect the quality of the spermatozoa penetrating the cumulus mass. Fertil. Steril. 92:971-978.

Hunter RH. 1996. Ovarian control of very low sperm/egg ratios at the commencement of mammalian fertilisation to avoid polyspermy. Mol. Reprod. Dev. 44:417-422.

Ikawa M, Inoue N, Benham AM and Okabe M. 2010. Fertilization: A sperm's journey to and interaction with the oocyte. J. Clin. Invest. 120:984-994.

Ikawa M, Nakanishi T, Yamada S, Wada I, Kominami K, Tanaka H, Nozaki M, Nishimune Y and Okabe M. 2001. Calmegin is required for fertilin alpha/beta heterodimerization and sperm fertility. Dev. Biol. 240:254-261.

Ilangumaran S and Hoessli DC. 1998. Effects of cholesterol depletion by cyclodextrin on the sphingolipid microdomains of the plasma membrane. Biochem. J. 335 (Pt 2):433-440.

Inoue N and Okabe M. 2008. Sperm-egg fusion assay in mammals. Methods Mol. Biol. 475:335-345.

Inoue N, Ikawa M, Isotani A and Okabe M. 2005. The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature 434:234-238.

Jaiswal BS, Cohen-Dayag A, Tur-Kaspa I and Eisenbach M. 1998. Sperm capacitation is, after all, a prerequisite for both partial and complete acrosome reaction. FEBS Lett. 427:309-313.

Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y, Sakagami J, Tada N, Miyazaki S and Kudo A. 2000. The gamete fusion process is defective in eggs of Cd9-deficient mice. Nat. Genet. 24:279-282.

Kang W, Zhou C, Koga Y and Baba T. 2010. Hyaluronan-degrading activity of mouse sperm hyaluronidase is not required for fertilization? J. Reprod. Dev. 56:140-144.

Kato Y, Shoei S and Nagao Y. 2010. Capacitation status of activated bovine sperm cultured in media containing methyl-beta-cyclodextrin affects the acrosome reaction and fertility. Zygote 19:21-30.

Kawano N, Kang W, Yamashita M, Koga Y, Yamazaki T,

Hata T, Miyado K and Baba T. 2010. Mice lacking two sperm serine proteases, ACR and PRSS21, are subfertile, but the mutant sperm are infertile *in vitro*. Biol. Reprod. 83: 359-369.

Kierszenbaum AL. 2000. Fusion of membranes during the acrosome reaction: A tale of two SNAREs. Mol. Reprod. Dev. 57:309-310.

Kim E, Baba D, Kimura M, Yamashita M, Kashiwabara S and Baba T. 2005. Identification of a hyaluronidase, Hyal5, involved in penetration of mouse sperm through cumulus mass. Proc. Natl. Acad. Sci. USA. 102:18028-18033.

Kim E, Yamashita M, Kimura M, Honda A, Kashiwabara S and Baba T. 2008. Sperm penetration through cumulus mass and zona pellucida. Int. J. Dev. Biol. 52:677-682.

Kim E, Yamashita M, Nakanishi T, Park KE, Kimura M, Kashiwabara S and Baba T. 2006. Mouse sperm lacking ADAM1b/ADAM2 fertilin can fuse with the egg plasma membrane and effect fertilization. J. Biol. Chem. 281: 5634-5639.

Kimura M, Kim E, Kang W, Yamashita M, Saigo M, Yamazaki T, Nakanishi T, Kashiwabara S and Baba T. 2009. Functional roles of mouse sperm hyaluronidases, HYAL5 and SPAM1, in fertilization. Biol. Reprod. 81: 939-947.

Kohno N, Yamagata K, Yamada S, Kashiwabara S, Sakai Y and Baba T. 1998. Two novel testicular serine proteases, TESP1 and TESP2, are present in the mouse sperm acrosome. Biochem. Biophys. Res. Commun. 245:658-665.

Kondoh G, Tojo H, Nakatani Y, Komazawa N, Murata C, Yamagata K, Maeda Y, Kinoshita T, Okabe M, Taguchi R and Takeda J. 2005. Angiotensin-converting enzyme is a GPI-anchored protein releasing factor crucial for fertilization. Nat. Med. 11:160-166.

Kreil G. 1995. Hyaluronidases-a group of neglected enzymes. Protein Sci. 4:1666-1669.

Kurasawa S, Schultz RM and Kopf GS. 1989. Egg-induced modifications of the zona pellucida of mouse eggs: Effects of microinjected inositol 1,4,5-trisphosphate. Dev. Biol. 133: 295-304.

Lai YM, Chang FH, Lee CL, Lee JD, Huang HY, Wang ML, Chan PJ, Chang MY and Soong YK. 1996. Coculture of human spermatozoa with reproductive tract cell monolayers can enhance sperm functions better than coculture with Vero cell monolayers. J. Assist. Reprod. Genet. 13:417-422.

Langlais J, Kan FW, Granger L, Raymond L, Bleau G and Roberts KD. 1988. Identification of sterol acceptors that stimulate cholesterol efflux from human spermatozoa during *in vitro* capacitation. Gamete Res. 20:185-201.

Lavy G, Boyers SP and DeCherney AH. 1988. Hyaluronidase removal of the cumulus oophorus increases *in vitro* fertilization. J. *In Vitro* Fert. Embryo Transf. 5:257-260.

Lefievre L, Conner SJ, Salpekar A, Olufowobi O, Ashton P, Pavlovic B, Lenton W, Afnan M, Brewis IA, Monk M, Hughes DC and Barratt CL. 2004. Four zona pellucida glycoproteins are expressed in the human. Hum. Reprod. 19:1580-1586.

Lin Y, Mahan K, Lathrop WF, Myles DG and Primakoff P. 1994. A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg. J. Cell Biol. 125: 1157-1163.

Liu C, Litscher ES, Mortillo S, Sakai Y, Kinloch RA, Stewart CL and Wassarman PM. 1996. 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. 93:5431-5436.

Maximov A, Tang J, Yang X, Pang ZP and Sudhof TC. 2009. Complexin controls the force transfer from SNARE complexes to membranes in fusion. Science 323:516-521.

Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, Suzuki K, Kosai K, Inoue K, Ogura A, Okabe M and Mekada E. 2000. Requirement of CD9 on the egg plasma membrane for fertilization. Science 287:321-324.

Muro Y, Buffone MG, Okabe M and Gerton GL. 2011. Function of the acrosomal matrix: Zona pellucida 3 receptor (ZP3R/sp56) is not essential for mouse fertilization. Biol. Reprod. in press.

Myles DG and Primakoff P. 1997. Why did the sperm cross the cumulus? To get to the oocyte. Functions of the sperm surface proteins PH-20 and fertilin in arriving at, and fusing with, the egg. Biol. Reprod. 56:320-327.

Nakagata N, Okamoto M, Ueda O and Suzuki H. 1997. Positive effect of partial zona-pellucida dissection on the *in vitro* fertilizing capacity of cryopreserved C57BL/6J transgenic mouse spermatozoa of low motility. Biol. Reprod. 57:1050-1055.

Ohmura K, Kohno N, Kobayashi Y, Yamagata K, Sato S, Kashiwabara S and Baba T. 1999. A homologue of pancreatic trypsin is localized in the acrosome of mammalian sperm and is released during acrosome reaction. J. Biol. Chem. 274:29426-29432.

Olson SD, Suarez SS and Fauci LJ. 2011. Coupling bioche-

mistry and hydrodynamics captures hyperactivated sperm motility in a simple flagellar model. J. Theor. Biol. 283: 203-216.

Oren-Benaroya R, Orvieto R, Gakamsky A, Pinchasov M and Eisenbach M. 2008. The sperm chemoattractant secreted from human cumulus cells is progesterone. Hum. Reprod. 23:2339-2345.

Patrat C, Serres C and Jouannet P. 2000. The acrosome reaction in human spermatozoa. Biol. Cell 92:255-266.

Primakoff P, Hyatt H and Myles DG. 1985. A role for the migrating sperm surface antigen PH-20 in guinea pig sperm binding to the egg zona pellucida. J. Cell Biol. 101:2239-2244.

Primakoff P, Lathrop W, Woolman L, Cowan A and Myles D. 1988. Fully effective contraception in male and female guinea pigs immunized with the sperm protein PH-20. Nature 335:543-546.

Rankin T, Familari M, Lee E, Ginsberg A, Dwyer N, Blanchette-Mackie J, Drago J, Westphal H and Dean J. 1996. Mice homozygous for an insertional mutation in the Zp3 gene lack a zona pellucida and are infertile. Development 122:2903-2910.

Rankin T, Talbot P, Lee E and Dean J. 1999. Abnormal zonae pellucidae in mice lacking ZP1 result in early embryonic loss. Development 126:3847-3855.

Rankin TL, O'Brien M, Lee E, Wigglesworth K, Eppig J and Dean J. 2001. Defective zonae pellucidae in Zp2-null mice disrupt folliculogenesis, fertility and development. Development 128:1119-1126.

Reim K, Mansour M, Varoqueaux F, McMahon HT, Sudhof TC, Brose N and Rosenmund C. 2001. Complexins regulate a late step in Ca^{2+}-dependent neurotransmitter release. Cell 104:71-81.

Rodger JC and Bedford JM. 1982. Separation of sperm pairs and sperm-egg interaction in the opossum, *Didelphis virginiana*. J. Reprod. Fertil. 64:171-179.

Rogers BJ and Morton BE. 1973. The release of hyaluronidase from capacitating hamster spermatozoa. J. Reprod. Fertil. 35:477-487.

Roggero CM, De Blas GA, Dai H, Tomes CN, Rizo J and Mayorga LS. 2007. Complexin/synaptotagmin interplay controls acrosomal exocytosis. J. Biol. Chem. 282:26335-26343.

Rubinstein E, Ziyyat A, Prenant M, Wrobel E, Wolf JP, Levy S, Le Naour F and Boucheix C. 2006. Reduced fertility of female mice lacking CD81. Dev. Biol. 290:351-358.

Runge KE, Evans JE, He ZY, Gupta S, McDonald KL, Stahlberg H, Primakoff P and Myles DG. 2007. Oocyte CD9 is enriched on the microvillar membrane and required for normal microvillar shape and distribution. Dev. Biol. 304: 317-325.

Saling PM and Storey BT. 1979. Mouse gamete interactions during fertilization *in vitro*. Chlortetracycline as a fluorescent probe for the mouse sperm acrosome reaction. J. Cell Biol. 83:544-555.

Snider DR and Clegg ED. 1975. Alteration of phospholipids in porcine spermatozoa during *in vivo* uterus and oviduct incubation. J. Anim. Sci. 40:269-274.

Sosnik J, Miranda PV, Spiridonov NA, Yoon SY, Fissore RA, Johnson GR and Visconti PE. 2009. Tssk6 is required for Izumo relocalization and gamete fusion in the mouse. J. Cell Sci. 122:2741-2749.

Spiridonov NA, Wong L, Zerfas PM, Starost MF, Pack SD, Paweletz CP and Johnson GR. 2005. Identification and characterization of SSTK, a serine/threonine protein kinase essential for male fertility. Mol. Cell. Biol. 5:4250-4261.

Stein KK, Primakoff P and Myles D. 2004. Sperm-egg fusion: Events at the plasma membrane. J. Cell Sci. 117:6269-6274.

Suarez SS. 1996. Hyperactivated motility in sperm. J. Androl. 17:331-335.

Suzuki K, Eriksson B, Shimizu H, Nagai T and Rodriguez-Martinez H. 2000. Effect of hyaluronan on monospermic penetration of porcine oocytes fertilized *in vitro*. Int. J. Androl. 23:13-21.

Talbot P, Geiske C and Knoll M. 1999. Oocyte pickup by the mammalian oviduct. Mol. Biol. Cell 10:5-8.

Tanghe S, Van Soom A, Nauwynck H, Coryn M and de Kruif A. 2002. Minireview: Functions of the cumulus oophorus during oocyte maturation, ovulation, and fertilization. Mol. Reprod. Dev. 61:414-424.

Tanigawa M, Miyamoto K, Kobayashi S, Sato M, Akutsu H, Okabe M, Mekada E, Sakakibara K, Miyado M, Umezawa A and Miyado K. 2008. Possible involvement of CD81 in acrosome reaction of sperm in mice. Mol. Reprod. Dev. 75:150-155.

Tanphaichitr N, Carmona E, Bou Khalil M, Xu H, Berger T and Gerton GL. 2007. New insights into sperm-zona pellucida interaction: Involvement of sperm lipid rafts. Front. Biosci. 12:1748-1766.

Tardif S, Wilson MD, Wagner R, Hunt P, Gertsenstein M, Nagy A, Lobe C, Koop BF and Hardy DM. 2010. Zonadhesin is

essential for species specificity of sperm adhesion to the egg zona pellucida. J. Biol. Chem. 285:24863-24870.

Tesarik J. 1989. Appropriate timing of the acrosome reaction is a major requirement for the fertilizing spermatozoon. Hum. Reprod. 4:957-961.

Travert C, Carreau S and Galeraud-Denis I. 2009. [*In vitro* capacitation]. Gynecol. Obstet. Fertil. 37:523-528.

Valencia A, Wens MA, Merchant H, Reyes R and Delgado NM. 1984. Capacitation of human spermatozoa by heparin. Arch. Androl. Suppl. 12:109-113.

van Gestel RA, Brewis IA, Ashton PR, Brouwers JF and Gadella BM. 2007. Multiple proteins present in purified porcine sperm apical plasma membranes interact with the zona pellucida of the oocyte. Mol. Hum. Reprod. 13:445-454.

van Gestel RA, Brewis IA, Ashton PR, Helms JB, Brouwers JF and Gadella BM. 2005. Capacitation-dependent concentration of lipid rafts in the apical ridge head area of porcine sperm cells. Mol. Hum. Reprod. 11:583-590.

van Gestel RA, Helms JB, Brouwers JF and Gadella BM. 2005. Effects of methyl-beta-cyclodextrin-mediated cholesterol depletion in porcine sperm compared to somatic cells. Mol. Reprod. Dev. 72:386-395.

Vergara GJ, Irwin MH, Moffatt RJ and Pinkert CA. 1997. *In vitro* fertilization in mice: Strain differences in response to superovulation protocols and effect of cumulus cell removal. Theriogenology 47:1245-1252.

Wang WH, Abeydeera LR, Fraser LR and Niwa K. 1995. Functional analysis using chlortetracycline fluorescence and *in vitro* fertilization of frozen-thawed ejaculated boar spermatozoa incubated in a protein-free chemically defined medium. J. Reprod. Fertil. 104:305-313.

Yamaguchi R, Muro Y, Isotani A, Tokuhiro K, Takumi K, Adham I, Ikawa M and Okabe M. 2009. Disruption of ADAM3 impairs the migration of sperm into oviduct in mouse. Biol. Reprod. 81:142-146.

Yamaguchi R, Yamagata K, Ikawa M, Moss SB and Okabe M. 2006. Aberrant distribution of ADAM3 in sperm from both angiotensin-converting enzyme (Ace)- and calmegin (Clgn)-deficient mice. Biol. Reprod. 75:760-766.

Yanagimachi R and Usui N. 1974. Calcium dependence of the acrosome reaction and activation of guinea pig spermatozoa. Exp. Cell Res. 89:161-174.

Zhang L, Jiang S, Wozniak PJ, Yang X and Godke RA. 1995. Cumulus cell function during bovine oocyte maturation, fertilization, and embryo development *in vitro*. Mol. Reprod. Dev. 40:338-344.

Zhao L, Burkin HR, Shi X, Li L, Reim K and Miller DJ. 2007. Complexin I is required for mammalian sperm acrosomal exocytosis. Dev. Biol. 309:236-244.

(접수: 2011. 11. 28 / 심사: 2011. 11. 28 / 채택: 2011. 12. 5)