Factors controlling sperm migration through the oviduct revealed by gene-modified mouse models

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Abstract: Mammalian fertilization is comprised of many steps including sperm survival in the uterus, sperm migration in the female reproductive tract, physiological and morphological changes to the spermatozoa, and sperm-egg interaction in the oviduct. In vitro studies have revealed essential factors for these fertilization steps for over half a century. However, the molecular mechanism of fertilization has recently been revised by the emergence of genetically modified animals. Here, we focus on essential factors for sperm fertilizing ability and describe recent advances in our knowledge of the mechanisms of mammalian fertilization, especially of sperm migration from the uterus into the oviduct.

Key words: female reproductive tract, fertilization, male infertility, sperm motility, uterotubal junction

Introduction

The human body is comprised of 60 trillion cells that originate from a fertilized egg produced by the fusion of a spermatozoon with an egg. Mammalian spermatozoa are morphologically differentiated in the testis, but freshly ejaculated spermatozoa are incapable of fertilization. Ejaculated spermatozoa gain their fertilizing ability in the female reproductive tract [2, 18]. The process that renders spermatozoa competent to fertilize an egg is called sperm capacitation [3]. These facts led to the possibility of performing in vitro fertilization (IVF) by mixing capacitated spermatozoa with eggs [20, 106]. In the past, factors important for fertilization were discovered via IVF experiments using biochemical approaches. However, recent studies using gene knockout (KO) methods have revealed that several sperm factors identified from the IVF system are not essential for in vivo fertilization [37, 76]. KO experiments have also unexpectedly revealed essential factors for fertilization in vivo. These essential factors play a role in spermatozoa gaining their fertilizing ability at stages such as spermatogenesis and epididymal transit. In this review, we focus on the molecular mechanism of the sperm fertilizing ability in the female reproductive tract revealed by in vivo analysis of genetically modified (GM) mice (Fig. 1).

Sperm Survival in the Uterus

Mammalian spermatozoa must travel a long distance from the uterus to the oviduct, where fertilization takes place. Ejaculated spermatozoa gain their fertilizing ability after remaining in the female reproductive tract for a period of time. This process is called sperm capacitation and is specific to mammalian spermatozoa. Though over 60 years have passed since the discovery of capacitation [3], its molecular mechanism remains to be fully determined [8]. Sperm surface-bound glycoproteins, CD52, CD55, and CD59, transferred from epididymis...
didymal luminal fluids were believed to protect spermatozoa from immunological attacks in the female reproductive tract [51]. Moreover, removal of these glycosylphosphatidylinositol-anchored proteins (GPI-APs) was also thought to induce sperm capacitation [13]. However, KO mouse experiments showed that CD52, CD55, CD59a, and CD59b are not essential for sperm fertilizing ability [34, 83, 91, 103]. Recently, Cd55b was identified in mice, but its physiological function has not been clarified.

Ejaculated spermatozoa mix together with a fluid, called seminal plasma, secreted from a male accessory sexual gland. Capacitated spermatozoa reversibly lose their fertilizing ability when treated with seminal plasma [19]. This finding suggests that seminal plasma contains a decapacitation factor that prevents sperm capacitation [11]. However, this phenomenon has been observed only in in vitro experiments that mixed capacitated spermatozoa with seminal plasma or candidate decapacitation factors [58, 61, 74]. Seminal vesicles, which secrete the main component of seminal plasma, contribute to reproduction because removal of seminal vesicles causes a significant reduction in male fertility [78]. Seminal vesicle protein secretion 2 (SVS2) is a major component of the seminal vesicle secretions, and it was found to be a decapacitation factor for mouse spermatozoa in vitro [1, 47]. Male Svs2 KO mice were severely subfertile because of a deficiency in copulatory plug formation and uterus-derived cytotoxicity that damaged the intrauterine spermatozoa [45]. Thus, the authors concluded that SVS2 protects ejaculated spermatozoa from immunological attack in the uterus and is required for spermatozoa to survive in the female reproductive tract. However, the decapacitation functions remain to be determined. Other functions of seminal fluid include influencing the growth and health of offspring [15]. Mammalian seminal plasma proteins may also have a key role in both fertilization and embryo development in vivo [64].

**Sperm Migration through the Uterotubal Junction**

The next obstacle for spermatozoa that survived in the uterus is passage through the uterotubal junction (UTJ) into the oviduct. The UTJ is the connection between the uterus and oviduct and is characterized as the distal portion of the oviduct. Although the structure of the UTJ varies among mammals, the passageway is usually narrow. In mice, the number of spermatozoa that pass through the UTJ is significantly reduced before reaching the oviduct ampulla. It has been proposed that most spermatozoa swim up through the UTJ into the oviduct by self-propulsion. However, KO mice experiments indicate that sperm motility alone is insufficient for sperm migration through the UTJ [37, 77]. There are more than 10 factors found to be essential for UTJ migration. In the following section, we discuss the interactions of these
factors and the current understanding of the mechanism of UTJ migration (Fig. 2).

Testis-specific ADAM proteins

A disintegrin and metalloproteinase (ADAM) family members are membrane-anchored metalloproteinases, and they regulate various events such as cell migration, cell adhesion, and cell interactions [86]. Testicular ADAMs, ADAM1B and ADAM2, heterodimerize to form fertilin. Fertilin is localized to the sperm plasma membrane and has been characterized as a sperm-egg fusion protein [12]. As expected, male Adam2 KO mice were found to be sterile [21], but the phenotype was not related to sperm-egg fusion [72]. When ADAM1B, a subunit of the fertilin heterodimer, was knocked out, both ADAM1B and ADAM2 disappeared from mature spermatozoa, but the mice were fully fertile [49]. Further investigations indicated that ADAM2 functions to form a dimer with ADAM1A in the endoplasmic reticulum (ER) of spermatogenic cells, leading to the localization of ADAM3 on the sperm surface [73]. Since male Adam3 KO mice were found to be sterile because of impaired sperm migration through the UTJ [87, 102], ADAM3 is thought to play a pivotal role in sperm migration through the UTJ. More than 10 proteins involved in sperm migration through the UTJ interact with ADAM3, affecting the protein amount and/or the localization of spermatozoa (Table 1).

Testis-specific ER chaperones

ADAM3 is a cysteine-rich, glycosylated membrane protein that is co-translationally translocated into the ER of spermatids, where numerous molecular chaperones and catalysts promote glycoprotein folding as well as the disposal of misfolded proteins. Membrane-bound calnexin (CANX) and soluble calreticulin (CALR) were originally found as homologous lectin chaperones that mainly mediate nascent glycoprotein folding in somatic cells. Testicular germ cell-specific homologues of CANX and CALR are calmegin (CLGN) and calsperrin (CALR3), respectively. CLGN mediates the heterodimerization of ADAM1A/ADAM2 that is required for the maturation of ADAM3 [38]. CALR3 binds directly to ADAM3 and regulates its maturation. Both Clgn and Calr3 KO mice lack ADAM3 in sperm and are sterile [39, 40]. Other chaperones, such as those in the protein disulfide isom-
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Erase (PDI) family proteins, have also been implicated in the intra- and intermolecular disulfide bond formation in the ER [79]. Among this protein family, PDIA3 is associated with CaNX/CaLr and contributes to the quality control cycle of newly synthesized glycoproteins in the ER. Testis-specific PDI-like protein, PDILT, co-operates with CaLr3 in testicular germ cells and plays an indispensable role in disulfide bond formation and folding of aDaM3 [95, 97]. Male Pdilt KO mice are infertile because of impaired transport of aDaM3 to the sperm surface [95]. Testicular germ cell-specific ER chaperones are essential for the folding and maturation of aDaM3.

**Testis-specific GPI-anchored proteins**

GPI-APs are anchored to the outer cell membrane by GPI and are critical at various points in mammalian fertilization [26, 52]. The GPI-AP complex, which consists of testis expressed gene 101 (TEX101) and lymphocyte antigen 6 complex locus k (LY6k), is present only in testicular germ cells, and it disappears from epididymal spermatozoa [54, 107]. TEX101 and LY6K are required for sperm migration into the oviduct [28, 29] (Fig. 3). Our study revealed that the transient interaction of the LY6K/TEX101 GPI-AP complex with ADAM3 is a critical step for ADAM3 maturation. Intriguingly, dissociation of the complex from ADAM3 is mediated by the GPI-AP releasing (GPIase) activity of angiotensin-converting enzyme (ACE). ACE is a well-characterized carboxy dipeptidase that regulates blood pressure. Ace-deficient mice showed low blood pressure, and the male mice were sterile [30, 53]. *In vitro* analysis demonstrated that TEX101 (but not LY6K) is the specific substrate for not only wild-type ACE but also zinc peptidase-defective ACE. These findings are consistent with the aberrantly remaining TEX101/LY6k protein complex on Ace KO mouse spermatozoa [28, 29]. As a result, ADAM3 dislocates from the Triton X-114 detergent-enriched phase to the detergent-depleted phase in Ace KO mouse spermatozoa, although ADAM3 localizes to both phases in wild-type mouse spermatozoa [104]. Therefore, ACE-mediated shedding of the GPI-AP complex, TEX101 and LY6K, is required for the correct localization of ADAM3 in epididymal spermatozoa and subsequent sperm fertilizing ability. The release of GPI-APs is one of the key events in activation of the sperm fertilizing ability [26].

**Essential factors regulating sperm migration through the UTJ**

To date, more than 10 factors have been reported to be essential for sperm migration through the UTJ (Table 1) and to be involved in ADAM3 maturation; however, there is no direct evidence that ADAM3 functions on the sperm surface during UTJ migration. Considering that ADAM3 is a pseudogene in humans, the contribution of undiscovered novel factors should be taken into account.

### Table 1. KO mouse lines with impaired sperm-ZP binding *in vitro* and impaired sperm migration from uterus into the oviduct

| Gene   | Expression pattern | Localization | In vitro sperm-ZP binding ability | Sperm migration ability to oviduct | Localization of ADAM3 on spermatozoa | Human ortholog | References                      |
|--------|--------------------|--------------|----------------------------------|-----------------------------------|--------------------------------------|----------------|---------------------------------|
| Ace-t  | Sperm              | Sperm surface| Impaired                         | Impaired                          | Aberrantly localized                 | +              | Krege JH, et al. 1995; Yamaguchi R, et al. 2006 |
| Adam1a | Testis             | Endoplasmic reticulum | Impaired                         | Impaired                          | Disappeared                          | –              | Nishimura H, et al. 2004        |
| Adam2  | Sperm              | Sperm surface | Impaired                         | Impaired                          | Disappeared                          | +              | Cho C, et al. 1998              |
| Adam3  | Sperm              | Sperm surface | Impaired                         | Impaired                          | Disappeared                          | –              | Shamsadin R, et al. 1999; Yamaguchi R, et al. 2009 |
| Calr3  | Testis             | Endoplasmic reticulum | Impaired                         | Impaired                          | Disappeared                          | +              | Ikawa M, et al. 2011            |
| Clgn   | Testis             | Endoplasmic reticulum | Impaired                         | Impaired                          | Disappeared                          | +              | Ikawa M, et al. 1997            |
| Ly6k   | Testis             | Testicular germ cell | Impaired                         | Impaired                          | Localized                            | +              | Fujihara Y, et al. 2014         |
| Pdilt  | Testis             | Endoplasmic reticulum | Impaired                         | Impaired                          | Disappeared                          | +              | Tokuhito K, et al. 2012         |
| Pgap1  | Ubiquitous         | Endoplasmic reticulum | Impaired                         | Impaired                          | Localized                            | +              | Ueda Y, et al. 2007             |
| Pmis2  | Sperm              | Sperm surface | Impaired                         | Impaired                          | Disappeared                          | –              | Yamaguchi R, et al. 2012         |
| Ppr37  | Testis             | Testicular germ cell | Impaired                         | Impaired                          | Disappeared                          | +              | Shen C, et al. 2013             |
| Rnase10 | Epididymis         | Epididymis    | Impaired                         | Impaired                          | Disappeared                          | +              | Krutskikh A, et al. 2012        |
| Tex101 | Testis             | Testicular germ cell | Impaired                         | Impaired                          | Disappeared                          | +              | Fujihara Y, et al. 2013         |
| Tpst2  | Ubiquitous         | Golgi apparatus | Impaired                         | Impaired                          | Disappeared                          | +              | Marcello M, et al. 2011         |

Essential factors regulating sperm migration through the UTJ

To date, more than 10 factors have been reported to be essential for sperm migration through the UTJ (Table 1) and to be involved in ADAM3 maturation; however, there is no direct evidence that ADAM3 functions on the sperm surface during UTJ migration. Considering that ADAM3 is a pseudogene in humans, the contribution of undiscovered novel factors should be taken into account.
This idea is also supported by the fact that ADAM3 localized normally in migration-defective Ly6k and Pgap1 KO spermatozoa [28, 96]. Because both LY6K and PGAP1 disappear during epididymal sperm maturation, these molecules do not directly function during UTJ migration. Recently, we identified sperm membrane proteins missing in Adam3 KO spermatozoa and found that protein missing in infertile sperm 2 (PMIS2) is a novel sperm protein required for UTJ migration [101]. Although many molecules have proven to be essential for sperm migration through the UTJ, the sperm migration mechanism per se is still unclear. Interestingly, migration-defective spermatozoa also show impaired binding to the zona pellucida (ZP) [77]. It is also reported that Adam3 KO spermatozoa are less adhesive than wild-type spermatozoa [87, 102]. To understand these defects, wild-type and Clgn KO chimeric mice were produced to test the migration ability of mixed spermatozoa. Although control wild-type spermatozoa could pass through the uterotubal junction (UTJ), the mixed wild-type spermatozoa could not compensate for the inability of Clgn KO spermatozoa to migrate into the oviduct [71]. These data implicate that there is an initial interaction with the UTJ entrance that may be a critical step prior to sperm migration into the oviduct. Further study is needed to resolve the mystery of the factor(s) controlling sperm migration into the oviduct.

**Contribution of sperm motility**

Sperm motility is also important for the UTJ passage. For example, male mice that lack cation channel, sperm associated 1 (CATSPER1), a component of a Ca^{2+} chan-
nel localized in the principle piece, were infertile due to impaired sperm motility [84]. Migration of *Cat sper1* KO spermatozoa through the UTJ was inefficient as observed by transillumination [33] and fluorescence microscopy [22]. Some *Cat sper1* KO spermatozoa were observed in the oviduct isthmus a few hours after coitus but disappeared with time. This suggests that the *Cat sper1* KO spermatozoa can pass through the UTJ but that most of the ejaculated spermatozoa in the uterus lose their motility with time before entering the UTJ. Mice lacking protein phosphatase 3 catalytic subunit gamma (PPP3CC), a catalytic subunit of calcineurin localized in the sperm tail, are another example illustrating the importance of sperm motility for UTJ passage [68]. *Ppp3cc* KO spermatozoa showed a rigid midpiece (Fig. 4A), and when the oviduct isthmus was observed two hours after copulation, less KO spermatozoa were observed compared with the control (Fig. 4B). Although *Ppp3cc* KO spermatozoa showed impaired motility, their velocity parameters in a hybrid background were comparable with those in a wild-type C57BL/6 background. Further studies are required to understand the exact role

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**Fig. 4.** Waveform and migration of *Ppp3cc* KO spermatozoa. (A) Flagellar movement patterns. Sperm motility was videotaped at 200 frames per second. Single frames throughout one beating cycle are superimposed. The midpiece (black arrow) is rigid in the spermatozoa obtained from *Ppp3cc* KO mice [68]. (B) Visualization of ejaculated spermatozoa in the female reproductive tract two hours post coitus (p.c.). Fewer *Ppp3cc* KO spermatozoa were observed in the oviduct isthmus.
of sperm motility in sperm migration, but flagellar movement patterns such as midpiece flexibility should also be taken into account for UTJ passage.

Recently, Muro et al. observed sperm migration in the female reproductive tract [70] using fluorescent spermatozoa that transgenically expressed green fluorescent protein (GFP) in the acrosome and red fluorescent protein from Discosoma sp. (DsRed2) in the midpiece [31]. They observed that the tail of spermatozoa migrating in the intramural UTJ seemed to be motionless. One explanation for this observation is that the midpiece that can be observed by DsRed2 fluorescence is motionless but that the principle piece and endpiece that cannot be observed with fluorescence are motile and play a role in sperm migration. However, analysis of Ppp3cc KO mice suggests that midpiece motility may in fact be important for UTJ passage [68]. Spermatozoa may pass through the UTJ with the midpiece “seemingly motionless” because of the viscous environment as suggested by Muro et al. [70].

Sperm Migration in the Oviduct

Sperm motility and hyperactivation for sperm migration

Once spermatozoa pass through the UTJ, they need to migrate through the oviduct to the ampulla. Sperm motility may be important for efficient sperm migration in the oviduct, as less Catsper1 and Ppp3cc KO spermatozoa were observed in the oviduct ampulla [22, 68]. A simple explanation for this importance is that spermatozoa swim by self-protrusion in the oviduct. However, there are several studies showing complex interactions between spermatozoa and the oviduct. Chang and Suarez observed that mouse spermatozoa attached to and detached from the epithelium of the oviduct isthmus [17], suggesting that spermatozoa may bind and unbind several times as they migrate through the oviduct. Detachment of spermatozoa may be caused by hyperactivated motility [17] characterized by a high amplitude and asymmetrical beating pattern of the sperm tail. It is interesting to mention that both Catsper1 and Ppp3cc KO spermatozoa cannot exhibit hyperactivated motility [16, 68]. Molecules that mediate the interaction between spermatozoa and the oviduct have not been identified yet using KO mouse models. In bulls, attachment of spermatozoa to the epithelium is mediated by binder of sperm protein 1 (BSP1), which is secreted by seminal vesicles [36]. There are two homologs of BSP in mice, Bsp1l and Bsp2 [57]; however, analyses of KO mice that lack these genes have not been performed.

Peristatic movement and sperm migration

Using fluorescent spermatozoa, Muro et al. observed that spermatozoa moved back and forth together with peristatic movement in the oviduct isthmus [70], suggesting that oviduct contractions may play a role in sperm migration. Ishikawa et al. observed a similar movement of sperm assemblage as well [42]. They showed that this movement was blocked and that fewer spermatozoa were found in the oviduct ampulla when peristatic movement was inhibited by the anticholinergic drug Padrin, suggesting that peristatic movement plays a role in sperm migration in the oviduct. However, a few spermatozoa can still reach the oviduct ampulla even with Padrin administration [42]. Another study that used Nicardipine to block oviduct contractions also showed that the spermatozoa could still reach the first loop of the oviduct isthmus or the oviduct ampulla [17]. These studies indicate that sperm motility may play a larger role in sperm migration in the oviduct isthmus rather than peristatic movement.

Oviductal fluid flow and sperm migration

How spermatozoa orient themselves in the oviduct remains an unanswered question. Miki and Clapham showed that mouse and human spermatozoa tend to swim against the flow (rheotaxis) and suggested that rheotaxis against oviductal flow is a major determinant of sperm guidance in the oviduct [65]. This is supported by an observation that Catsper1 KO spermatozoa cannot exhibit rheotaxic behavior and cannot migrate through the oviduct efficiently. In addition to rheotaxis, chemotaxis [60, 88] and thermotaxis [7] are also implicated in sperm migration. Because these hypotheses are based on in vitro studies, further in vivo experiments are necessary to understand how spermatozoa understand direction in the oviduct.

Interaction between Spermatozoa and Cumulus Cells

Sperm enzymes involved in sperm passage through cumulus cell layers

Spermatozoa move into the ampulla of the oviduct and encounter the cumulus-cell oocyte complex (COC). The COC consists of ovulated eggs covered by an ex-
tracellular matrix (ECM), the ZP, and a cumulus cell layer filled with hyaluronic acid (Figs 5A and B). Mouse spermatozoa have at least two hyaluronidases, sperm adhesion molecule 1 (SPAM1) and hyaluronoglucosaminidase 5 (HYAL5) [48]. SPAM1 was first identified as a sperm receptor for the ZP and was later reported to have hyaluronidase activity that enables spermatozoa to pass thorough the COC [59, 80, 81]. While Spam1 KO mice are fertile, Spam1 KO spermatozoa show a reduced ability to disperse cumulus cells in vitro [4]. Hyal5 KO mice are fertile both in vitro and in vivo [50], suggesting functional redundancies in these genes.

Proteinase activity is also implicated in COC penetration as shown below. Acrosin (ACR) and protease, serine 21 (PRSS21), are trypsin-like serine proteases and are localized on the sperm head [6, 35]. Although these sperm proteases were thought to play an essential role in ZP binding and penetration, Acr KO spermatozoa were fertile, albeit with a slight delay in ZP penetration in vitro [5]. Prss21 KO mouse lines were also fully fertile in vivo [105]. Moreover, Acr and Prss21 double KO mice were subfertile because of impaired sperm penetration through the cumulus matrix and ZP in vitro [46]. This indicates that the sperm trypsin-like activity is not essential for in vivo fertilization in mice. Therefore, the sperm factor required for penetration through the cumulus matrix in vivo remains to be determined.

COC factors that modulate sperm functions

Sperm chemotaxis is found in not only marine invertebrates but also mammals [44]. Chemoattractants are present in oviductal fluid and are also secreted from the COC [24]. In humans, the COC secretes sperm chemoattractants after ovulation [90]. Progesterone secreted from

Fig. 5. Sperm passage through the cumulus cell layer and the ZP. (A) The cumulus-cell oocyte complex (COC). Ovulated eggs are covered by a cumulus cell layer and the zona pellucida (ZP). (B) Sperm passage through the cumulus cell layer. The spermatozoa penetrating through the cumulus cell layer were observed using the red fluorescence localized in the midpiece. The egg is highlighted with a white dotted line. (C) Rescue of ZP penetration failure using glutathione (GSH). When GSH is used, the ZP is destabilized and expanded. Ppp3cc KO spermatozoa can penetrate the ZP in the presence of GSH and fertilize the egg. Black arrows indicate the pronuclei.
the COC influences several functions including hyperactivation and the acrosome reaction in human spermatozoa [9]. The extranuclear-mediated effects of progesterone stimulate an influx of calcium, tyrosine phosphorylation of proteins, and other signaling molecules [82]. Progesterone-induced calcium influx is mediated by a sperm-specific calcium channel CATSPER in human spermatozoa [60, 88]. It has recently been revealed that human CATSPER activation by progesterone is triggered by the steroid binding to a serine hydrolase, abhydrolase domain containing 2 (ABHD2) [66]. Furthermore, the CATSPER channel complex may serve as a polymodal sensor for multiple chemicals (odorants, 8-Br-cNMPs, or menthol) [14]. However, mouse *Catsper* does not react to progesterone induction *in vitro*, and mouse *Abhd2* is not essential for male fertility [69]. Since the sequence similarity of *Catsper* orthologs is low (less than 50%) [85], the mechanism of sperm calcium entry may differ in each species.

Prostaglandin E2 (PGE2) is a key mediator of ovulation [23]. One of the four subtypes of PGE2 receptor, prostaglandin E receptor 2 (*Ptger2*), is expressed in the cumulus cells. Female *Ptger2* KO mice are severely subfertile due to impaired cumulus expansion in the oviduct [32]. PGE2-PTGER2 signaling facilitates cumulus ECM assembly and sperm passage through cumulus cell layers. The gene expression profile indicates that cumulus cells upregulate a set of immune response- and chemokine-related genes during ovulation [93]. One of the chemokines, chemokine ligand 7 (*Ccl7*), is overexpressed abnormally in *Ptger2* KO cumulus cells, and excessive cumulus ECM assembly interferes with sperm migration through the COC [94]. These results suggest that CCL7 promotes cumulus ECM assembly to protect the oocyte and functions as a chemoattractant for spermatozoa. While it is unclear why this occurs, proper interaction between prostaglandin and chemokine signaling is required for successful fertilization [89]. Further studies have shown that CCL7 facilitates sperm migration towards the COC *in vitro*. Recently, we found that *Adam3* KO spermatozoa are able to fertilize cumulus-intact eggs but not cumulus-free eggs [95]. The supernatant of cumulus cells is able to partially restore *Adam3* KO sperm fertilizing ability. These data also suggest that COC factors can modulate sperm fertilizing ability.

**Sperm Penetration through the ZP**

During *in vitro* fertilization, numerous spermatozoa bind to the ZP, and it has been long believed that ZP binding ability is critical for sperm fertilizing ability. However, when *Adam3* KO spermatozoa, which cannot bind to the ZP [87], were deposited directly into the oviduct to circumvent sperm migration through the UTJ, the ovulated eggs were fertilized [95]. These data questioned the importance of ZP binding ability.

There are two possible factors that are necessary for sperm penetration through the ZP, namely, proteases and sperm motility. Proteases were thought to be important because the acrosome contains proteases that are released during the acrosome reaction. However, ACR and PRSS21, trypsin-like serine proteases in the sperm head, are not essential for male fertility in mice, as mentioned previously [5, 46, 105]. Further, recent live-imaging studies demonstrated that mouse spermatozoa underwent the acrosome reaction before contact with the ZP [43, 56, 70], and rabbit and mouse spermatozoa that penetrated the ZP once could penetrate the ZP again and fertilize ZP-intact eggs [41, 55]. These results suggest that the proteases that are released from the acrosome are not necessary for ZP penetration. However, careful interpretation is required because there is a possibility that proteases remain attached to the sperm head after the acrosome reaction, and this may contribute to ZP penetration.

There is positive evidence that sperm motility is crucial for ZP penetration. Field vole spermatozoa can penetrate through the ZP of mice and hamsters without the acrosome reaction [98], suggesting that the mechanical force generated by sperm motility is important for ZP penetration. This idea is supported by *Catsper1* or *Ppp3cc* KO mice that exhibit impaired sperm motility and failure to penetrate the ZP [68, 84]. Both *Catsper1* and *Ppp3cc* KO spermatozoa do not exhibit hyperactivated motility as mentioned previously. However, *Ppp3cc* KO spermatozoa could penetrate through the ZP when the eggs were treated with glutathione (GSH), which reduces disulfide bonds and destabilizes the ZP (Fig. 5C) [10, 68, 92]. The fertilized eggs developed to term when they were transplanted into the oviduct of pseudopregnant mice. This method using GSH in IVF could be useful to further identify factors that are involved in ZP penetration.
Use of GM animals is a powerful approach to clearly identify the in vivo function(s) of a given gene. In mammalian fertilization research especially, most findings based on biochemical in vitro approaches have been revised by the analyses of KO mouse models [37, 77]. However, the conventional KO method is expensive, laborious, and time-consuming to perform. Recently, the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system has emerged as a genome editing tool in mice, rats, and other animal models [99]. This system enables researchers to make GM mice easier and quicker than the conventional KO method [100]. We have also established a method to generate GM mice using a CRISPR/Cas9 expression plasmid [25, 62, 63] and have analyzed reproductive phenotypes of GM mice using this method [27, 67, 75, 108]. Reproductive biology is one of the most suitable research fields that can use GM animals. We therefore believe that mutant animals will soon unravel whole gene functions through gene-disruption experiments.

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References

1. Araki, N., Trencsényi, G., Krasznai, Z.T., Nizsalóczki, E., Sakamoto, A., Kawano, N., Miyado, K., Yoshida, K., and Yoshida, M. 2015. Seminal vesicle secretion 2 acts as a protectant of sperm sterols and prevents ectopic sperm capactitation in mice. Biol. Reprod. 92: 8. [Medline] [CrossRef]
2. Austin, C.R. 1951. Observations on the penetration of the sperm in the mammalian egg. Aust. J. Sci. Res., B 4: 581–596. [Medline] [CrossRef]
3. Austin, C.R. 1952. The capacitation of the mammalian sperm. Nature 170: 326. [Medline] [CrossRef]
4. 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. [Medline] [CrossRef]
5. 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. [Medline] [CrossRef]
6. Baba, T., Kashiwabara, S., Watanabe, K., Itoh, H., Michikawa, Y., Kimura, K., Takada, M., Fukamizu, A., and Ariy, Y. 1989. Activation and maturation mechanisms of boar acrosin zymogen based on the deduced primary structure. J. Biol. Chem. 264: 11920–11927. [Medline] [CrossRef]
7. Bahat, A., Tur-Kaspa, I., Gakamsky, A., Gijalas, L.C., Brubart, H., and Eisenbach, M. 2003. Thermotaxis of mammalian sperm cells: a potential navigation mechanism in the female genital tract. Nat. Med. 9: 149–150. [Medline] [CrossRef]
8. Bailey, J.L. 2010. Factors regulating sperm capactitation. Syst Biol Reprod Med 56: 334–348. [Medline] [CrossRef]
9. Baldi, E., Luconi, M., Muratori, M., Marchiani, S., Tamburino, L., and Forti, G. 2009. Nongenomic activation of spermatozoa by steroid hormones: facts and fictions. Mol. Cell. Endocrinol. 308: 39–46. [Medline] [CrossRef]
10. Bath, M.L. 2010. Inhibition of in vitro fertilizing capacity of cryopreserved mouse sperm by factors released by damaged sperm, and stimulation by glutathione. PLoS One 5: e9387. [Medline] [CrossRef]
11. Bedford, J.M. and Chang, M.C. 1962. Removal of decapacitation factor from seminal plasma by high-speed centrifugation. Am. J. Physiol. 202: 179–181. [Medline]
12. Blobel, C.P., Wolfsberg, T.G., Turck, C.W., Myles, D.G., Prima, P., and White, J.M. 1992. A potential fusion peptide and an integrin ligand domain in a protein active in sperm-egg fusion. Nature 356: 248–252. [Medline] [CrossRef]
13. Boerke, A., van der Lit, J., Loliaco, F., Stout, T.A., Helms, J.B., and Gadella, B.M. 2014. Removal of GPI-anchored membrane proteins causes clustering of lipid microdomains in the apical head area of porcine sperm. Theriogenology 81: 613–624. [Medline] [CrossRef]
14. Brenker, C., Goodwin, N., Weyand, I., Kashikar, N.D., N curse, M., Krähling, M., Müller, A., Kaupp, U.B., and Strünkner, T. 2012. The CatSper channel: a polymodal chemosensor in human sperm. EMBO J. 31: 1654–1665. [Medline] [CrossRef]
15. Bromfield, J.J., Schjenken, J.E., Chin, P.Y., Care, A.S., Jasper, M.J., and Robertson, S.A. 2014. Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. Proc. Natl. Acad. Sci. USA 111: 2200–2205. [Medline] [CrossRef]
16. Carlson, A.E., Westenbroek, R.E., Quill, T., Ren, D., Clapham, D.E., Hille, B., Garbers, D.L., and Babcock, D.F. 2003. CatSper1 required for evoked Ca2+ entry and control of flagellar function in sperm. Proc. Natl. Acad. Sci. USA 100: 14864–14868. [Medline] [CrossRef]
17. Chang, H. and Suarez, S.S. 2012. Unexpected flagellar
movement patterns and epithelial binding behavior of mouse sperm in the oviduct. Biol. Reprod. 86: 140, 1–8. [Medline] [CrossRef]

18. Chang, M.C. 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature 168: 697–698. [Medline] [CrossRef]

19. Chang, M.C. 1957. A detrimental effect of seminal plasma on the fertilizing capacity of sperm. Nature 179: 258–259. [Medline] [CrossRef]

20. Chang, M.C. 1959. Fertilization of rabbit ova in vitro. Nature 184(Suppl 7): 466–467. [Medline] [CrossRef]

21. Cho, C., Bunch, D.O., Faure, J.E., Goulding, E.H., Eddy, E.M., Primakoff, P., and Myles, D.G. 1998. Fertilization defects in sperm from mice lacking fertilin beta. Science 281: 1857–1859. [Medline] [CrossRef]

22. Chun, J.J., Shim, S.H., Everley, R.A., Gygi, S.P., Zhuang, X., and Clapham, D.E. 2014. Structurally distinct Ca(2+) signaling domains of sperm flagella orchestrate tyrosine phosphorylation and motility. Cell 157: 808–822. [Medline] [CrossRef]

23. Duffy, D.M. 2015. Novel contraceptive targets to inhibit ovulation: the prostaglandin E2 pathway. Hum. Reprod. Update 21: 652–670. [Medline] [CrossRef]

24. Eisenbach, M. and Giojalas, L.C. 2006. Sperm guidance in developmental, and reproductive biology. J. Biol. Chem. 281: 18428–18435. [Medline] [CrossRef]

25. Fujihara, Y. and Ikawa, M. 2014. CRISPR/Cas9-based genome editing in mice by single plasmid injection. Methods Enzymol. 546: 319–336. [Medline] [CrossRef]

26. Fujihara, Y. and Ikawa, M. 2016. GPI-AP release in cellular, developmental, and reproductive biology. J. Lipid Res. 57: 538–545. [Medline] [CrossRef]

27. Fujihara, Y., Oji, A., Larasati, T., Kojima-Kita, K., and Ikawa, M. 2017. Human Globozoospermia-Related Gene Spata16 Is Required for Sperm Formation Revealed by CRISPR/Cas9-Mediated Mouse Models. Int. J. Mol. Sci. 18: 18. [Medline] [CrossRef]

28. Fujihara, Y., Okabe, M., and Ikawa, M. 2014. GPI-anchored protein complex, LY6K/TEX101, is required for sperm migration into the oviduct and male fertility in mice. Biol. Reprod. 90: 60. [Medline] [CrossRef]

29. Fujihara, Y., Tokuhiro, K., Muro, Y., Kondoh, G., Araki, Y., Ikawa, M., and Okabe, M. 2013. Expression of TEX101, regulated by ACE, is essential for the production of fertile mouse spermatozoa. Proc. Natl. Acad. Sci. USA 110: 8111–8116. [Medline] [CrossRef]

30. Hagaman, J.R., Moyer, J.S., Bachman, E.S., Sibony, M., Magyar, P.L., Welch, J.E., Smithies, O., Krege, J.H., and O’Brien, D.A. 1998. Angiotensin-converting enzyme and male fertility. Proc. Natl. Acad. Sci. USA 95: 2552–2557. [Medline] [CrossRef]

31. Hasuwa, H., Muro, Y., Ikawa, M., Kato, N., Tsujimoto, Y., and Okabe, M. 2010. Transgenic mouse sperm that have green acrosome and red mitochondria allow visualization of sperm and their acrosome reaction in vivo. Exp. Anim. 59: 105–107. [Medline] [CrossRef]

32. Hizaki, H., Segi, E., Sugimoto, Y., Hirose, M., Saji, T., Usuki, F., Matsuoka, T., Noda, Y., Tanaka, T., Yoshida, N., Narumiya, S., and Ichikawa, A. 1999. Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP(2). Proc. Natl. Acad. Sci. USA 96: 10501–10506. [Medline] [CrossRef]

33. Ho, K., Wolff, C.A., and Suarez, S.S. 2009. CatSper-null mutant spermatozoa are unable to ascend beyond the oviductal reservoir. Reprod. Fertil. Dev. 21: 345–350. [Medline] [CrossRef]

34. Holt, D.S., Botto, M., Bygrave, A.E., Hanna, S.M., Walport, M.J., and Morgan, B.P. 2001. Targeted deletion of the CD59 gene causes spontaneous intravascular hemolysis and hemoglobinuria. Blood 98: 442–449. [Medline] [CrossRef]

35. Honda, A., Yamagata, K., Sugiuira, 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. [Medline] [CrossRef]

36. Ignotz, G.G., Lo, M.C., Perez, C.L., Gwathmey, T.M., and Suarez, S.S. 2001. Characterization of a fucose-binding protein from bull sperm and seminal plasma may be responsible for formation of the oviductal sperm reservoir. Biol. Reprod. 64: 1806–1811. [Medline] [CrossRef]

37. Ikawa, M., Inoue, N., Benham, A.M., and Okabe, M. 2010. Fertilization: a sperm’s journey to and interaction with the oocyte. J. Clin. Invest. 120: 984–994. [Medline] [CrossRef]

38. Ikawa, M., Nakashishi, T., Yamada, S., Wada, I., Kominami, K., Tanaka, H., Nozaki, M., Nishimune, Y., and Okabe, M. 2001. Calmodulin is required for fertilin alpha/beta heterodimerization and sperm fertilization. Dev. Biol. 240: 254–261. [Medline] [CrossRef]

39. Ikawa, M., Tokuhiro, K., Yamaguchi, R., Benham, A.M., Tamura, T., Wada, I., Satoh, Y., Inoue, N., and Okabe, M. 2011. Calserpin is a testis-specific chaperone required for sperm fertility. J. Biol. Chem. 286: 5639–5646. [Medline] [CrossRef]

40. Ikawa, M., Wada, I., Kominami, K., Watanabe, D., Toshimori, K., Nishimune, Y., and Okabe, M. 1997. The putative chaperone calmodin is required for sperm fertility. Nature 387: 607–611. [Medline] [CrossRef]

41. Inoue, N., Satoh, Y., Ikawa, M., Okabe, M., and Yanagimachi, R. 2011. Acrosome-reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs. Proc. Natl. Acad. Sci. USA 108: 20008–20011. [Medline] [CrossRef]

42. Ishikawa, Y., Usui, T., Yamashita, M., Kanemori, Y., and Baba, T. 2016. Surfing and Swimming of Ejaculated Sperm in the Mouse Oviduct. Biol. Reprod. 94: 89. [Medline] [CrossRef]

43. Jin, M., Fujiwara, E., Kakiuchi, Y., Okabe, M., Satoh, Y., Baba, S.A., Chiba, K., and Hirohashi, N. 2011. Most fertilizing mouse spermatozoa bind their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc. Natl. Acad. Sci. USA 108: 4892–4896. [Medline] [CrossRef]

44. Kaupp, U.B., Kashkar, N.D., and Weyand, I. 2008. Mechanisms of sperm chemotaxis. Annu. Rev. Physiol. 70: 93–117. [Medline] [CrossRef]

45. Kawano, N., Araki, N., Yoshida, K., Hibino, T., Ohnami, N.,
Makino, M., Kanai, S., Hasuwa, H., Yoshida, M., Miyado, K., and Umezawa, A. 2014. Seminal vesicle protein SVS2 is required for sperm survival in the uterus. Proc. Natl. Acad. Sci. USA 111: 4145–4150. [Medline] [CrossRef]

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 infertilize in vitro. Biol. Reprod. 83: 359–369. [Medline] [CrossRef]

Kawano, N. and Yoshida, M. 2007. Semen-coagulating protein, SVS2, in mouse seminal plasma controls sperm fertility. Biol. Reprod. 76: 353–361. [Medline] [CrossRef]

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. [Medline] [CrossRef]

Kim, E., Yamashita, M., Nakanishi, T., Park, K.E., Kimura, M., Kashiwabara, S., and Baba, T. 2009. Functional roles of mouse sperm hyaluronidases, HYAL5 and SPAM1, in fertilization. Biol. Reprod. 81: 939–947. [Medline] [CrossRef]

Kirchhoff, C. and Hale, G. 1996. Cell-to-cell transfer of glycosylphosphatidylinositol-anchored membrane proteins during sperm maturation. Mol. Hum. Reprod. 2: 177–184. [Medline] [CrossRef]

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. [Medline] [CrossRef]

Krege, J.H., John, S.W., Langenbach, L.L., Hodgın, J.B., Hagaman, J.R., Bachman, E.S., Jennette, J.C., O’Brien, D.A., and Smithies, O. 1995. Male-female differences in fertility and blood pressure in ACE-deficient mice. Nature 375: 146–148. [Medline] [CrossRef]

Kurita, A., Takizawa, T., Takayama, T., Totsukawa, K., Matsubara, S., Shibahara, H., Orgebin-Crist, M.C., Sendo, F., Shinkai, Y., and Araki, Y. 2001. Identification, cloning, and initial characterization of a novel mouse testicular germ cell-specific antigen. Biol. Reprod. 64: 935–945. [Medline] [CrossRef]

Kuzan, F.B., Fleming, A.D., and Seidel, G.E. Jr. 1984. Successful fertilization in vitro of fresh intact oocytes by perivitelline (acrosome-reacted) spermatozoon of the rabbit. Fertil. Steril. 41: 766–770. [Medline] [CrossRef]

La Spina, F.A., Puga Molina, L.C., Romarowski, A., Vitale, A.M., Falzone, T.L., Kraaf, D., Hirohashi, N., and Buffone, M.G. 2016. Mouse sperm begin to undergo acrosomal exocytosis in the upper isthmus of the oviduct. Dev. Biol. 411: 172–182. [Medline] [CrossRef]

Lefèvre, J., Fan, J., Chevalier, S., Sullivan, R., Carmona, E., and Manjunath, P. 2007. Genomic structure and tissue-specific expression of human and mouse genes encoding homologues of the major bovine seminal plasma proteins. Mol. Hum. Reprod. 13: 45–53. [Medline] [CrossRef]

Lin, M.H., Lee, R.K., Hwu, Y.M., Lu, C.H., Chu, S.L., Chen, Y.J., Chang, W.C., and Li, S.H. 2008. SPINKL1, a Kazal-type serine protease inhibitor-like protein purified from mouse seminal vesicle fluid, is able to inhibit sperm capacitation. Reproduction 136: 559–571. [Medline] [CrossRef]

Lin, Y., Mahan, K., Lathrop, W.F., Myles, D.G., 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. [Medline] [CrossRef]

Lishko, P.V., Botchkina, I.L., and Kirichok, Y. 2011. Progestosterone activates the principal Ca2+ channel of human sperm. Nature 471: 387–391. [Medline] [CrossRef]

Lu, C.H., Lee, R.K., Hwu, Y.M., Chu, S.L., Chen, Y.J., Chang, W.C., Lin, S.P., and Li, S.H. 2011. SERPIN2, a serine protease inhibitor extensively expressed in adult male mouse reproductive tissues, may serve as a murine sperm decapacitation factor. Biol. Reprod. 84: 514–525. [Medline] [CrossRef]

Mashiko, D., Fujihara, Y., Satouh, Y., Miyata, H., Isotani, A., and Ikawa, M. 2013. Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. Sci. Rep. 3: 3355. [Medline] [CrossRef]

Mashiko, D., Young, S.A., Muto, M., Kato, H., Nozawa, K., Ogawa, M., Noda, T., Kim, Y.J., Satouh, Y., Fujihara, Y., and Ikawa, M. 2014. Feasibility for a large scale mouse mutagenesis by injecting CRISPR/Cas plasmid into zygotes. Dev. Growth Differ. 56: 122–129. [Medline] [CrossRef]

McGraw, L.A., Suarez, S.S., and Wolfner, M.F. 2015. On a matter of seminal importance. BioEssays 37: 142–147. [Medline] [CrossRef]

Miki, K. and Clapham, D.E. 2013. Rheotaxis guides mammalian sperm. Curr. Biol. 23: 443–452. [Medline] [CrossRef]

Miller, M.R., Mannowitz, N., Iavaroni, A.T., Safavi, R., Gracheva, E.O., Smith, J.F., Hill, R.Z., Bautista, D.M., Kirichok, Y., and Lishko, P.V. 2016. Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. Science 352: 555–559. [Medline] [CrossRef]

Miyata, H., Castaneda, J.M., Fujihara, Y., Yu, Z., Archambault, D.R., Isotani, A., Kiyozumi, D., Kriseman, M.L., Mashiko, D., Matsumura, T., Matzuk, R.M., Mori, M., Noda, T., Oji, A., Okabe, M., Prunskate-Hyyrylainen, R., Ramirez-Solis, R., Satouh, Y., Zhang, Q., Ikawa, M., and Matzuk, M.M. 2016. Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. Proc. Natl. Acad. Sci. USA 113: 7704–7710. [Medline] [CrossRef]

Miyata, H., Satouh, Y., Mashiko, D., Muto, M., Nozawa, K., Shiba, K., Fujihara, Y., Isotani, A., Inaba, K., and Ikawa, M. 2015. Sperm calcineurin inhibition prevents mouse fertility with implications for male contraceptive. Science 350: 442–445. [Medline] [CrossRef]

Miyata, K., Oike, Y., Hoshii, T., Maekawa, H., Ogawa, H.,
Suda, T., Araki, K., and Yamamura, K. 2005. Increase of smooth muscle cell migration and of intimal hyperplasia in mice lacking the alpha/beta hydrolase domain containing 2 gene. Biochem. Biophys. Res. Commun. 329: 296–304. [Medline] [CrossRef]

70. Muro, Y., Hasuwa, H., Isotani, A., Miyata, H., Yamagata, K., Ikawa, M., Yanagimachi, R., and Okabe, M. 2016. Behavior of Mouse Spermatooza in the Female Reproductive Tract soon after Mating to the Beginning of Fertilization. Biol. Reprod. 94: 80. [Medline] [CrossRef]

71. Nakamishi, T., Isotani, A., Yamaguchi, R., Ikawa, M., Baba, T., Suarez, S.S., and Okabe, M. 2004. Selective passage through the uterotubal junction of sperm from a mixed population produced by chimeras of calmodin-knockout and wild-type male mice. Biol. Reprod. 71: 959–965. [Medline] [CrossRef]

72. Nishimura, H., Cho, C., Branciforte, D.R., Myles, D.G., and Primakoff, P. 2001. Analysis of loss of adhesive function in sperm lacking cysteintin or fertilin beta. Dev. Biol. 233: 204–213. [Medline] [CrossRef]

73. Nishimura, H., Kim, E., Nakamishi, T., and Baba, T. 2004. Possible function of the ADAM1α/ADAM2 Fertilin complex in the appearance of ADAM3 on the sperm surface. J. Biol. Chem. 279: 34957–34962. [Medline] [CrossRef]

74. Nixon, B., MacIntyre, D.A., Mitchell, L.A., Gibbs, G.M., O’Bryan, M., and Aitken, R.J. 2006. The identification of mouse sperm-surface-associated proteins and characterization of their ability to act as decapacitation factors. Biol. Reprod. 74: 275–287. [Medline] [CrossRef]

75. Okabe, M. 2015. Mechanisms of fertilization elucidated by gene targeting. Proc. Natl. Acad. Sci. USA 112: 1782–1787. [Medline] [CrossRef]

76. Okabe, M. 2013. The cell biology of mammalian fertilization. Mol. Biol. Reprod. 94: 80. [Medline] [CrossRef]

77. Okabe, M., Segi-Nishida, E., Okuno, Y., Tsujimoto, G., Narumiya, S., and Sugimoto, Y. 2010. Expression profiling of cumulus cells reveals functional changes during ovulation and central roles of prostaglandin EP2 receptor in cAMP signaling. Biochimie 92: 665–675. [Medline] [CrossRef]

78. Peitz, B. and Olds-Clarke, P. 1986. Effects of seminal vesicle removal on fertility and uterine sperm motility in the house mouse. Biol. Reprod. 35: 608–617. [Medline] [CrossRef]

79. Persson, S., Rosenquist, M., Knoblach, B., Khosravi-Far, R., Sommerin, M., and Michalak, M. 2005. Diversity of the protein disulfide isomerase family: identification of breast tumor induced Hag2 and Hag3 as novel members of the protein family. Mol. Phylogenet. Evol. 36: 734–740. [Medline] [CrossRef]

80. Phelps, B.M., Primakoff, P., Koppel, D.E., Low, M.G., and Myles, D.G. 1988. Restricted lateral diffusion of PH-20, a PI-anchored sperm membrane protein. Science 240: 1780–1782. [Medline] [CrossRef]

81. 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. [Medline] [CrossRef]

82. Publicover, S., Harper, C.V., and Barratt, C. 2007. [Ca2+]i signalling in sperm--making the most of what you’ve got. Nat. Cell Biol. 9: 235–242. [Medline] [CrossRef]

83. Qin, X., Krumrei, N., Grubissich, L., Dobarro, M., Aktas, H., Perez, G., and Halperin, J.A. 2003. Deficiency of the mouse complement regulatory protein mCd59b results in spontaneous hemolytic anemia with platelet activation and progressive male infertility. Immunity 18: 217–227. [Medline] [CrossRef]

84. Ren, D., Navarro, B., Perez, G., Jackson, A.C., Hsu, S., Shi, Q., Tilly, J.L., and Clapham, D.E. 2001. A sperm ion channel required for sperm motility and male fertility. Nature 413: 603–609. [Medline] [CrossRef]

85. Ren, D. and Xia, J. 2010. Calcium signaling through CatSper channels in mammalian fertilization. Physiology (Bethesda) 25: 165–175. [Medline] [CrossRef]

86. Seals, D.F. and Courtineidge, S.A. 2003. The ADAMS family of metalloproteases: multidomain proteins with multiple functions. Genes Dev. 17: 7–30. [Medline] [CrossRef]

87. Shamsadin, R., Adham, I.M., Nayernia, K., Heinlein, U.A., Oberecker, H., and Engel, W. 1999. Male mice deficient for germ-cell cysteintin are infertile. Biol. Reprod. 61: 1445–1451. [Medline] [CrossRef]

88. Strünker, T., Goodwin, N., Brener, C., Kashikar, N.D., Weyand, I., Seifert, R., and Kaupp, U.B. 2011. The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm. Nature 471: 382–386. [Medline] [CrossRef]

89. Sugimoto, Y., Inazumi, T., and Tsuchiya, S. 2015. Roles of prostaglandin receptors in female reproduction. J. Biochem. 157: 73–80. [Medline] [CrossRef]

90. Sun, F., Bahat, A., Gakamsky, A., Girsh, E., Katz, N., Gijalas, L.C., Tur-Kaspa, I., and Eisenbach, M. 2005. Human sperm chemotaxis: both the oocyte and its surrounding cumulus cells secrete sperm chemotacticants. Hum. Reprod. 20: 761–767. [Medline] [CrossRef]

91. Sun, X., Funk, C.D., Deng, C., Sahu, A., Lambris, J.D., and Song, W.C. 1999. Role of decay-accelerating factor in regulating complement activation on the erythrocyte surface as revealed by gene targeting. Proc. Natl. Acad. Sci. USA 96: 628–633. [Medline] [CrossRef]

92. Takeo, T. and Nakagata, N. 2011. Reduced glutathione enhances fertility of frozen/thawed C57BL/6 mouse sperm after exposure to methyl-beta-cyclodextrin. Biol. Reprod. 85: 1066–1072. [Medline] [CrossRef]

93. Tamba, S., Yodoi, R., Morimoto, K., Inazumi, T., Suenko, M., Segi-Nishida, E., Okuno, Y., Tsujimoto, G., Narumiya, S., and Sugimoto, Y. 2010. Expression profiling of cumulus cells reveals functional changes during ovulation and central roles of prostaglandin EP2 receptor in cAMP signaling. Biochimie 92: 665–675. [Medline] [CrossRef]

94. Tamba, S., Yodoi, R., Segi-Nishida, E., Ichikawa, A., Narumiya, S., and Sugimoto, Y. 2008. Timely interaction between prostaglandin and chemokine signaling is a prerequisite for successful fertilization. Proc. Natl. Acad. Sci. USA 105: 14539–14544. [Medline] [CrossRef]

95. Tokuhiro, K., Ikawa, M., Benham, A.M., and Okabe, M. 2012. Protein disulfide isomerase homolog PDILT is required for quality control of sperm membrane protein ADAM3 and male fertility [corrected]. Proc. Natl. Acad. Sci. USA 109: 3850–3855. [Medline] [CrossRef]
96. Ueda, Y., Yamaguchi, R., Ikawa, M., Okabe, M., Morii, E., Maeda, Y., and Kinoshita, T. 2007. PGAP1 knock-out mice show otocephaly and male infertility. J. Biol. Chem. 282: 30373–30380. [Medline] [Crossref]

97. van Lith, M., Hartigan, N., Hatch, J., and Benham, A.M. 2005. PDILT, a divergent testis-specific protein disulfide isomerase with a non-classical SXXC motif that engages in disulfide-dependent interactions in the endoplasmic reticulum. J. Biol. Chem. 280: 1376–1383. [Medline] [Crossref]

98. Wakayama, T., Ogura, A., Suto, J., Matsubara, Y., Kurohmaru, M., Hayashi, Y., and Yanagimachi, R. 1996. Penetration by field vole spermatozoa of mouse and hamster zonae pellucidae without acrosome reaction. J. Reprod. Fertil. 107: 97–102. [Medline] [Crossref]

99. Wang, H., La Russa, M., and Qi, L.S. 2016. CRISPR/Cas9 in Genome Editing and Beyond. Annu. Rev. Biochem. 85: 227–264. [Medline] [Crossref]

100. Wang, H., Yang, H., Shivalila, C.S., Dawlaty, M.M., Cheng, A.W., Zhang, F., and Jaenisch, R. 2013. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153: 910–918. [Medline] [Crossref]

101. Yamaguchi, R., Fujihara, Y., Ikawa, M., and Okabe, M. 2012. Mice expressing aberrant sperm-specific protein PMIS2 produce normal-looking but fertilization-incompetent spermatozoa. Mol. Biol. Cell 23: 2671–2679. [Medline] [Crossref]

102. Yamaguchi, R., Muro, Y., Isotani, A., Tokuhiro, K., Taku-ki, 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. [Medline] [Crossref]

103. Yamaguchi, R., Yamagata, K., Hasuwa, H., Inano, E., Ikawa, M., and Okabe, M. 2008. Cd52, known as a major maturation-associated sperm membrane antigen secreted from the epididymis, is not required for fertilization in the mouse. Genes Cells 13: 851–861. [Medline] [Crossref]

104. Yamaguchi, R., Yamagata, K., Ikawa, M., Moss, S.B., and Okabe, M. 2006. Aberrant distribution of ADAM3 in sperm from both angiotensin-converting enzyme (Ace)- and calmcin (Clgn)-deficient mice. Biol. Reprod. 75: 760–766. [Medline] [Crossref]

105. Yamashita, M., Honda, A., Ogura, A., Kashiwabara, S., Fukami, K., and Baba, T. 2008. Reduced fertility of mouse epididymal sperm lacking Prss21/Tesp5 is rescued by sperm exposure to uterine microenvironment. Genes Cells 13: 1001–1013. [Medline] [Crossref]

106. Yanagimachi, R. and Chang, M.C. 1963. Fertilization of Hamster Eggs in Vitro. Nature 200: 281–282. [Medline] [Crossref]

107. Yoshitake, H., Tsukamoto, H., Maruyama-Fukushima, M., Takamori, K., Ogawa, H., and Araki, Y. 2008. TEX101, a germ cell-marker glycoprotein, is associated with lymphocyte antigen 6 complex locus k within the mouse testis. Biochem. Biophys. Res. Commun. 372: 277–282. [Medline] [Crossref]

108. Young, S.A., Miyata, H., Satouh, Y., Kato, H., Nozawa, K., Isotani, A., Aitken, R.J., Baker, M.A., and Ikawa, M. 2015. CRISPR/Cas9-Mediated Rapid Generation of Multiple Mouse Lines Identified Ccdc63 as Essential for Spermiogenesis. Int. J. Mol. Sci. 16: 24732–24750. [Medline] [Crossref]