Proteolysis in Reproduction: Lessons From Gene-Modified Organism Studies

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The physiological roles of proteolysis are not limited to degrading unnecessary proteins. Proteolysis plays pivotal roles in various biological processes through cleaving peptide bonds to activate and inactivate proteins including enzymes, transcription factors, and receptors. As a wide range of cellular processes is regulated by proteolysis, abnormalities or dysregulation of such proteolytic processes therefore often cause diseases. Recent genetic studies have clarified the inclusion of proteases and protease inhibitors in various reproductive processes such as development of gonads, generation and activation of gametes, and physical interaction between gametes in various species including yeast, animals, and plants. Such studies not only clarify proteolysis-related factors but the biological processes regulated by proteolysis for successful reproduction. Here the physiological roles of proteases and proteolysis in reproduction will be reviewed based on findings using gene-modified organisms.

Keywords: protease, fertilization, proteolysis, protease inhibitor, pseudoprotease, gene-modified animal models, ubiquitin-proteasome system, sperm maturation

INTRODUCTION

Although a simple peptide bond between two amino acids in water at room temperature has a half-life of several years (1), the hydrolysis of a peptide bond is significantly accelerated under the presence of proteases. As well as mediating non-specific protein hydrolysis, proteases also act as processing enzymes that perform highly selective, limited, and efficient cleavage of specific substrates. As many biological processes are influenced by this irreversible post-translational protein modification, dysregulation of the expression and/or function of proteases underlie many human pathological processes and have therefore been an intensely studied class of targets for drug discovery.

Abbreviations: ACE, angiotensin converting enzyme; ADAM, a disintegrin-like and metalloproteinase domain; ADAMTS, a disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif; CSN, constitutive photomorphogenic-9 signalosome; EMS, ethylmethane-sulfonate; GGT, glutamyltranspeptidase; I(±), inter-α-trypsin inhibitor; KI, knock-in; KO, knockout; OVCH2, ovochymase 2; S-Lap, sperm-Leucylaminopeptidase; SUMO, small ubiquitin-related modifier; TASP1, threonine aspartase 1; TMP, trimethylpsoralen; TNFα, tumor necrosis factor-α; UPS, ubiquitin-proteasome system; USP, ubiquitin-specific protease.
By searching *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans* genome databases with a gene ontology term “peptidase activity” (GO:0008233), 51, 506, and 448 genes encoding proteases, respectively, can be identified (2–4). In the mouse and human genome, 628 and 553 protease genes exist, respectively (5). In *Arabidopsis thaliana*, 723 protease genes were reported (6). Based on catalytic mechanisms, proteases can be divided into five classes: cysteine proteases, serine proteases, metalloproteases, threonine proteases, and aspartic proteases. After activation of the amide, cysteine, serine, and threonine proteases utilize the namesake residue to attack the amide carbonyl group, whereas metalloproteases and aspartic proteases use an activated water molecule as a nucleophile. As proteases bind their substrates between the substrate side chains and well-defined substrate-binding pockets within the active site, they have their own preference for substrate amino acid sequence proximal to the cleavage site (7). There are some enzymatically inactive pseudoproteases encoded in the mammalian genome in which the amino acid residues indispensable for catalytic activity are substituted. As proteases are potentially toxic, their activities are strictly regulated as such by pH, specific ion concentrations, posttranslational modifications, and spatiotemporal expression of protease inhibitors.

The contribution of proteases depends on their intracellular or extracellular localization where they act on substrate proteins. The ubiquitin-proteasome system (UPS) is a complex but sophisticated intracellular proteolytic system in eukaryotes; this complex system degrades unneeded or damaged proteins by proteolysis. When target proteins are post-translationally labeled with ubiquitin, a protein of 76-amino acid residues exhibiting high sequence conservation among eukaryotes, they will be recognized and degraded by the proteasome.

Proteolytic processing events are fundamental in reproductive processes including gametogenesis, fertilization, and embryonic development. Recent advances in generating gene-modified animals have identified many proteases and their regulators associated with reproduction in various species including yeast, invertebrates, vertebrates, and plants. In the following sections the physiological importance of proteolysis in reproduction will be overviewed based on findings obtained by gene-modified organism studies. Proteolysis-related genes essential in reproduction identified by gene-modified animal studies are listed in Table 1. Few proteins are known to be proteolytically processed under certain reproductive situations. They are, however, not included in this review as the physiological roles of such processing in reproduction are not fully clarified at present.

### UNICELLULAR ORGANISMS

**Saccharomyces cerevisiae**

*S. cerevisiae*, Baker’s yeast, is a model diploid unicellular organism. *S. cerevisiae* can stably exist as either a diploid or a haploid. When stressed, *S. cerevisiae* can undergo meiosis to produce four haploid spores. Haploid cells are capable of fusing with other haploid cells of the opposite mating type (an ‘α’ cell can only mate with an ‘α’ cell, and vice versa) to produce a stable diploid cell. a and α cells produce mating peptide pheromones a-factor and α-factor, respectively. Ste24p and Ax1lp encoded by ste24 and alx1, respectively, are metalloendopeptidases that process precursor peptide to produce mature mating a-factor pheromone (8, 9).

### MULTICELLULAR ORGANISMS

#### I: INVERTEBRATES

The body of multicellular organisms consists of two types of cells with different lineages, i.e., germ cells and somatic cells. Germ cells produce gametes for fertilization, whereas somatic cells develop reproductive organs to support gametogenesis and fertilization by germ cells. Therefore, dysfunction of proteolysis in either cell lineage can result in fertility defects.

**Nematodes**

*Caenorhabditis elegans* is androdioecious; i.e., it has two sexes, hermaphrodite and male, whereas *Ascaris suum* is dioecious, being either male or female. They develop two U-shaped gonads in which gametes are generated and fertilization occurs. Several proteases and inhibitors have been identified to regulate nematode reproductive processes.

Oogenesis and fertilization are affected when *cpi-2a*, encoding a cystatin-like cysteine protease inhibitor, is mutated (10). Nullification of *dss-1* encoding a 26S proteasome subunit provokes sterility because of deficient oogenesis (14). Knockdown of *puromycin-sensitive aminopeptidase* encoded by *pam-1* causes delayed oocyte maturation and subfertility (17). Deletion of *dpf-3* encoding a serine protease causes sterility because of impaired spermatogenesis (15). *gon-1* encoding a disintegrin-like and metalloproteinase domain with thrombospondin type 1 motif (ADAMTS) is necessary for morphogenesis of U-shaped gonads (11, 12). A mutant worm lacking * timp-1* encoding a tissue inhibitor of metalloproteinase also shows deficient gonadal development (13). A double mutant in which *sup-17* and *adm-4*, encoding nematode orthologs of mammalian membrane metalloproteases ADAM10 and ADAM17, respectively, are sterile because of aberrant spermathecal function (16). Unlike mammalian flagellated sperm, nematode sperm are amoeboid cells. For successful fertilization, sperm must be activated prior to contacting an oocyte in both *C. elegans* and *A. suum*. This sperm activation is called spermiogenesis through which round immobile spermatids transform into motile, fertilization-competent spermatoozoa. Mechanistically, spermiogenesis occurs by sensing extracellular signals and can be reproduced in *vitro* by exposing spermatids to proteases such as Pronase and proteinase K. A trypsin-like secreted protease encoded by *try-5* is expressed in the vas deferens and triggers activation of spermatids (18). *swm-1* encodes a secreted protein with a trypsin inhibitor-like domain, and *swm-1* mutant males...
| Gene   | Protein feature       | Protein localization | Gene-modified organism          | Fertility | Phenotype                                                      | Refs. |
|--------|-----------------------|----------------------|---------------------------------|-----------|----------------------------------------------------------------|-------|
| S. cerevisiae |                       |                      |                                 |           |                                                                |       |
| ste24  | Prenyl protein-        | Intracellular         | Ethylmethane-sulfonate (EMS) mutagenesis | Sterile   | MAT a-specific sterility.                                        | (8)   |
|         | specific endoprotease  | membrane             |                                 |           |                                                                |       |
| avl1   | Metalloprotease       | Intracellular         | UV exposure                     | Sterile   | Defect in a-factor pheromone secretion.                          | (9)   |
| C. elegans |                   |                      |                                 |           |                                                                |       |
| cpi-2a | Cystatin-like cysteine protease inhibitor | Extracellular |                                 | Sterile   | Oocyte-specific sterility.                                      | (10)  |
| gon-1  | Metalloprotease       | Extracellular         | EMS mutagenesis                 | Sterile   | Gonadal developmental defect.                                  | (11, 12) |
| tmp-1  | Metalloprotease       | Extracellular         | Trimethylpsoralen (TMP)–UV-mutagenesis | Sterile   | Gonadal growth defect.                                          | (13)  |
| dss-1  | 26S proteasome subunit| Intracellular         | Deletion mutant                 | Sterile   | Defects in oogenesis.                                           | (14)  |
| dpl-3 sup-17; adm-4 | Serine protease | Intracellular membrane |                                 | Sterile   | Impaired spermatogenesis.                                       | (15)  |
|         | ADAM metalloproteases |                      |                                 |           |                                                                |       |
| pam-1  | Metalloprotease       | Intracellular         | RNAi knockdown                  | Subfertility | Decreased brood size. Expanded pachytene.                      | (17)  |
| try-5  | Serine protease       | Extracellular         | Deletion mutant                 | Fertile   | try-5 functions in parallel to sper-8 for male fertility.      | (18)  |
| swm-1  | Trypsin inhibitor-like | Extracellular         | EMS mutagenesis                 | Reduced male fertility | Ectopic sperm activation within the male reproductive tract. Failure of sperm transfer to hermaphrodite. | (19)  |
| gona-1 | Metalloprotease       | Nucleus               | Deletion by CRISPR/Cas9          | Fertility defects | Decrease of fertility in later generations because of genomic instability | (20)  |
| T12E12.6 | Metalloprotease | Intracellular membrane |                                 | Subfertility | Decreased brood size.                                          | (17)  |
| zmp-2  | Metalloprotease       | Extracellular         | RNAi knockdown                  | Subfertility | Reduced offspring production.                                   | (21)  |
| D. melanogaster |                   |                      |                                 |           |                                                                |       |
| CG9000; CG9001; CG9002 | Yeast ste24p ortholog proteases | Intracellular membrane | Ends-out gene targeting | Male fertility defects | Abnormal spermatid maturation.                                  | (22)  |
| Prosalpha6T | Proteasome subunit | Intracellular         | KO                              | Male infertility | Spermatogenic defects in sperm individualization and nuclear maturation. | (23)  |
| Duba   | Deubiquitylating enzyme | Intracellular         | Imprecise P-element excision    | Male infertility | Defects in spermatid individualization.                         | (24)  |
| Dronc  | Cysteine protease     | Intracellular         | Transgenic expression of dominant-negative DRONC | Uncertain | Defects in spermatid individualization.                         | (25)  |
| Dredd  | Cysteine protease     | Intracellular         | EMS mutagenesis                 | Fertile   | Defects in spermatid individualization.                         | (25)  |
| Dark   | Caspase activator     | Intracellular         | Enhancer trap                   | Male infertility | Sperm were completely immotile.                                 | (26)  |
| Htra2  | Serine protease       | Mitochondria          | P element mobilization          | Male infertility | Defective spermatogenesis.                                     | (27)  |
| S-Lap1-8 | Leucylamino-peptidase | Intracellular         | EMS mutagenesis                 | Male infertility | Deficient accumulation of paracrystalline material in mitochondria. | (28)  |
| Sens   | Trypsin-like protease | Extracellular         | Classical mutant, CRISPR/Cas9 Knockdown | Male infertility or subfertility | Females laid fewer number of eggs when mated to Sens knockout males. Sperm remained in storage in the seminal receptacle. Mutant sperm are quickly discarded by females. | (29)  |
| Nep4   | Metalloprotease       | Cell membrane         | KO                              | Male infertility | Defective oogenesis.                                           | (30)  |
| Dcp-1  | Metalloprotease       | Intracellular         | KO                              | Female infertility | Female infertility                                               | (31)  |

(Continued)
### TABLE 1 | Continued

| Gene             | Protein feature | Protein localization | Gene-modified organism | Fertility    | Phenotype                                                                 | Refs. |
|------------------|-----------------|----------------------|------------------------|--------------|---------------------------------------------------------------------------|-------|
| mh               | Metalloprotease  | Nucleus              | EMS mutagenesis, P element mobilization | Female infertility | The integration of paternal chromosomes in the zygote was specifically affected. | \(32,\)  
| Ance             | Angiotensin-converting enzyme | Extracellular | EMS mutagenesis | Male infertility | Compound heterozygotes for two different lethal alleles are male sterile. | \(34\) |
| SfEc             | Serine protease  | Extracellular RNAi knockdown | Male infertility | Details are unknown. Females also show slightly decreased fertility. | \(35\) |
| ome              | Serine protease  | Cell membrane EMS mutagenesis | Male subfertility | Details are uncertain. | \(36\) |
| Mmp2             | Metalloprotease  | Extracellular RNAi | Female subfertility | Ovulation was blocked. | \(37\) |
| A. socius ejac-sp| Serine protease  | Extracellular RNAi knockdown | Male infertility | Reduced ability to induce a female to lay eggs. | \(38\) |
| B. mori Otp      | Serine protease  | Cell membrane KO | Female infertility | Mutant females laid fewer eggs than wild-type females and eggs did not hatch. | \(39\) |
| Ser2             | Serine protease  | Extracellular KO | Male infertility | Wild-type females mated with mutant males laid eggs normally but the eggs did not hatch. | \(40\) |
| S. litura Oap    | Serine protease  | Cell membrane KO | Female infertility | Mutant sperm morphology is normal but they do not enter eggs. | \(41\) |
| P. xylostella Ser2| Serine protease  | Extracellular KO | Male infertility | The growth, development, mating behavior, or egg laying was not affected. | \(42\) |
| B. dorsalis Bdcp-1| Cysteine protease | Intracellular RNAi knockdown, KO | Male infertility | Impaired ovary development. | \(43\) |
| M. musculus Pama| Proteasome component | Nucleus | KO | Male infertility | Arrested spermatogenesis at spermatocyte stage. | \(44\) |
| Psme3            | Proteasome activator | Intracellular | KO | Male subfertility | Decreased sperm number and motility. | \(45\) |
| Psme4            | Proteasome activator | Nucleus | KO | Severe male subfertility | Defective spermatogenesis. | \(46\) |
| Psme3; Psme4     | Proteasome activator | Intracellular Double KO | Male infertility | Morphologically normal sperm with motility defect. | \(47\) |
| Cops5            | Metalloprotease  | Intracellular | KO | Male infertility | Male infertility. Germ cells undergo significant apoptosis at a premeiotic stage. Defects in sperm motility. | \(48\) |
| Usp2             | Ubiquitin-specific protease | Nucleus | KO | Male subfertility | Apoptosis of spermatocytes. | \(49\) |
| Usp9k            | Ubiquitin-specific protease | Cytoplasm | Vasa-cre, Usp9k<sup>FY</sup> | Male infertility | Unsynapsed chromosomes in pachynema and defective chiasma formation in diplonema, apoptosis of metaphase spermatocytes and decrease of spermatids. | \(50,\)  
| Usp26            | Ubiquitin-specific protease | Intracellular | KO | Severe male subfertility | Impaired spermatogenesis. | \(51\) |
| Usp1             | Ubiquitin-specific protease | Nucleus | KO | Male infertility | Degeneration of spermatogonia resulting in the absence of sperm. | \(52\) |
| Apaf1            | Caspase activator | Intracellular | KO | Male infertility | Defective spermatogenesis | \(53\) |
| Agbi5            | Metalloprotease  | Intracellular | KO | Male infertility | Near devoid of sperm. | \(54\) |
| Gona             | Metalloprotease  | Nucleus | KO | Male infertility | Male infertility Release immature germ cells. | \(55\) |
| Tasp1            | Endopeptidase    | Nucleus | KO | Male infertility | Globozoosperma, no acrosomal cap. | \(56\) |
| Tysnd1           | Serine protease  | Peroxisome | KO | Male infertility | Oligoasthenoteratozoospermia in heterozygotes, azoospermia in homozygotes. | \(57\) |
| Spink2           | Serine protease inhibitor | Extracellular | KO | Male infertility | | \(58\) |
TABLE 1 | Continued

| Gene | Protein feature | Protein localization | Gene-modified organism | Fertility | Phenotype                                                                 | Refs. |
|------|----------------|----------------------|------------------------|-----------|---------------------------------------------------------------------------|-------|
| Serpin5 | Serine protease inhibitor | Extracellular | KO | Male infertility | Abnormal spermatogenesis due to destruction of the Sertoli cell barrier. | (60) |
| Adamts2 | Metalloproteinase | Extracellular | KO | Male infertility | Marked decrease in testicular sperm. | (61) |
| Acr | Serine protease | Acrosome | KO | Male infertility | Delayed fertilization. | (62, 63) |
| Pcsk4 | Serine protease | Acrosomal membrane | KO | Male infertility | Putatively due to impaired fertilization. | (64, 65) |
| Tmpras12 | Serine protease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (66, 67, 68) |
| Prss55 | Serine protease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (69) |
| Tryx5 | Serine protease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (70) |
| Prss37 | Metalloprotease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (71) |
| Ace | Metalloproteinase | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (72) |
| Adam1a | Pseudoprotease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (73) |
| Adam2 | Pseudoprotease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (74, 75) |
| Adam3 | Pseudoprotease | Plasma membrane | KO | Male infertility | Deficient sperm migration into oviduct. | (76) |
| Cst8; Cst9; Cst11; Cst12; Cst13; Cstdc1; Cstdc2; Cst1f | Cystatin-like inhibitor | Extracellular | Multiple KO | Male infertility | Deficient sperm migration into oviduct. | (77, 78) |
| Cpe | Metalloprotease | Extracellular | Spontaneous mutation | Male subfertility | Normal spermatogenesis but reduced egg fertilization. | (79) |
| Adam24 | Pseudoprotease | Plasma membrane | KO | Male subfertility | Mutant spermatozoa possessed decreased motility, angulated and curled tails, and fragile necks. Decreased in vitro zona pellucida binding and acrosome reaction. | (80, 81) |
| Adam7 | Pseudoprotease | Plasma membrane | KO | Male subfertility | Decreased cell height in caput epididymis, spermatogranuloma, kinked sperm flagellum and reduced sperm motility. | (82) |
| Cst3 | Cysteine protease inhibitor | Extracellular | KI (Leu68Gln) | Male subfertility | Reduced viability of spermatozoa and large agglutinated clumps. | (83) |
| Sertpine2 | Serine protease inhibitor | Extracellular | KO | Male subfertility | Inadequate semen coagulation and deficient vaginal plug formation upon copulation. | (84) |
| Tmpras6 | Serine protease | Plasma membrane | KO | Female infertility | Marked retardation in ovarian maturation. | (85) |
| Ambp | Serine protease inhibitor | Extracellular | KO | Female subfertility | Defective cumulus matrix expansion. | (86, 87) |
| Psen1 | Aspartic protease | Endoplasmic reticulum, Golgi, endosome, plasma membrane | KO | Female infertility | Primordial follicles near the ovarian cortex and consisting largely of ovarian stromal elements. | (88) |
| Adamts1 | Metalloprotease | Extracellular | KO | Female infertility | Fewer numbers of mature follicles in ovary, thick and convoluted uterus. | (89) |
| Lonp | Serine protease | Mitochondria | Gdf9-cre or Zp3-cre; Lonp1<sup>fr</sup> | Female infertility | Impaired follicular development, progressive oocyte death, ovarian reserve loss. | (90) |
| Furin | Serine protease | Golgi, endosome, plasma membrane, extracellular | Gdf9-cre or Zp3-cre; Furin<sup>fr</sup> | Female infertility | Arrest of early secondary follicles. | (91) |
| Pappa | Metalloprotease | Extracellular | KO | Female subfertility | Reduced litter size and reduced ovulatory capacity, probably because of decreased bioavailability of ovarian insulin-like growth factor. | (92) |
| Astl | Metalloprotease | Extracellular | KO | Female subfertility | No ZP2 cleavage after fertilization. | (93) |

(Continued)
| Gene         | Protein feature     | Protein localization | Gene-modified organism | Fertility                  | Phenotype                                                   | Refs.   |
|--------------|---------------------|----------------------|-------------------------|----------------------------|-------------------------------------------------------------|---------|
| Fetub        | Metalloprotease     | Extracellular        | KO                      | Female                     | Premature zona pellucida hardening.                         | (97)    |
| Serpinc1     | Serine protease     | Extracellular        | KI                      | Female                     | Thrombosis in placenta and penile vessels.                 | (98)    |
| Adam10       | Metalloprotease     | Cell membrane        | Tie2-cre; Adam10fl     | Female                     | Fifty percent of mutant females are infertile because of    | (100)   |
| Adamts18     | Metalloprotease     | Extracellular        | KO                      | Female                     | Fifty percent of mutant females are infertile because of    |         |
| Plg          | Serine protease     | Extracellular        | KO                      | Female                     | Fifty percent of mutant females are infertile because of    |         |
| Timp1        | Metalloprotease     | Extracellular        | KO                      | Female                     | Fifty percent of mutant females are infertile because of    |         |
| Pcsk2        | Serine protease     | Extracellular        | KO                      | Female                     | Fifty percent of mutant females are infertile because of    |         |
| Esp1l        | Cysteine protease   | Nucleus              | Meox2fl; Esp1fl-S1121A | Male infertility           | Spontaneous mutation, Male infertility                     | (105)   |
| Agtbp1       | Metalloprotease     | Intracellular        | Spontaneous mutation,  | Male infertility           | Poor development of secondary follicles into antral         | (109–112) |
| Clpp         | Serine protease     | Mitochondria         | KO                      | Male infertility           | Reduced testis and seminal vesicle size, reduced            | (113)   |
| Npepps       | Metalloprotease     | Nucleus, cytosol     | Gene trap               | Female infertility         | Poor development of secondary follicles into antral         | (114)   |
| Ggt1         | Protease            | Plasma membrane      | KO                      | Male infertility           | Reduced testis and seminal vesicle size, reduced            | (115)   |
| Immp2l       | Serine protease     | Mitochondria         | KO                      | Severe male infertility    | Reduced testis and seminal vesicle size, reduced            | (117)   |
| Adam17       | Metalloprotease     | Extracellular        | Sox9-cre; Adam17fl      | Male infertility           | Reduced testis and seminal vesicle size, reduced            | (118)   |

**Mesocricetus auratus**

- **Acr**
  - Serine protease
  - Acrosome
  - KO
  - Male infertility
  - Sperm failure in zona pellucida penetration.

- **R. norvegicus**
  - Adamts16
    - Metalloprotease
    - Extracellular
    - KO
    - Male infertility
    - Cryptorchidism.

- **D. rerio**
  - adamts9
    - Metalloprotease
    - Extracellular
    - KO
    - Female infertility
    - Ovary malformation.

- **H. sapiens**
  - SPINK2
    - Serine protease inhibitor
    - Extracellular
    - Spontaneous mutation
    - Male infertility
    - Azoospermia.

(Continued)
are infertile because of ectopic premature activation of sperm (19). Like in C. elegans, activation of spermatozoa by exposure to extrinsic protease \textit{in vitro} can also be seen in several insect species (132, 133). \textit{spe-4} encoding a presenilin, an aspartyl protease with intramembrane proteolytic activity prevents spermatid activation because \textit{spe-4} mutant males progress directly to functional spermatozoa without the need for an activation signal (134).

\textit{gcna-1} encodes nuclear metalloprotease. \textit{gcna-1} deletion causes genomic instability decreasing fertility in later generations (20). \textit{T12E12.6} encodes intracellular metalloprotease whereas \textit{zmp-2} encodes secreted metalloproteases. Knockdown of either of them results in reduced offspring production (17, 21).

**Insects**

The reproductive system of \textit{Drosophila melanogaster} is more complex compared with nematodes; it is composed of gonads, genital ducts, and accessory structures. Several proteases have been implicated in \textit{D. melanogaster} spermatogenesis. In the \textit{D. melanogaster} genome, there are five genes paralogous to \textit{S. cerevisiae} \textit{ste24} encoding a type I prenyl protease. Deletion of three tandemly arrayed \textit{ste24} paralogs results in male fertility defects manifesting late in spermatogenesis (22).

All \textit{Drosophila} spermatid nuclei descended from a primary spermatocyte remain connected to each other \textit{via} an extensive network of cytoplasmic bridges. Spermatids should therefore be physically dissociated from each other by a process referred to as sertolization and a ubiquitin-proteasome system regulates this process. Males in which \textit{Prosalpha8} \textit{D} encoding a testis-specific proteasome core particle subunit was ablated are sterile because of defects in sperm individualization and nuclear maturation (23). \textit{Duba} encodes a deubiquitylating enzyme and \textit{Dubal} null mutants are male sterile and display defects in spermatid individualization (24). The non-apoptotic function of caspases also contributes to individualization. DARK is a \textit{Drosophila} homolog of mammalian caspase activator Apaf-1, whereas \textit{DRONC} and \textit{DREDD} are \textit{Drosophila} apical caspases. Flies deficient in DARK or expressing a dominant-negative version of \textit{DRONC} failed individualization (25, 135). \textit{Dredd} null flies also often show individualization defects (25).

In \textit{D. melanogaster} sperm, mitochondrial derivatives run along the entire flagellum to provide structural rigidity for flagellar movement. Two mitochondrial derivatives (i.e., major and minor) differentiate and major one accumulates paracrystalline material by the end of spermatogenesis. S-Lap-null mutants are sterile because sperm are completely immotile (26), whereas spermatogenesis is defective in another \textit{Htra2} mutant line (27).
encoding a cysteine protease was ablated in their germline, the resulting females were infertile because of defective oogenesis (31).

Several proteases also of concern in Drosophila reproduction include maternal haploid or mh encodes the Drosophila homolog of SPRTN, a conserved metalloprotease essential for resolving DNA–protein cross-linked products. Paternal chromatids of mh mutants are unable to separate in the anaphase of the first embryonic mitosis and form a chromatin bridge. As a consequence, haploid nuclei of maternal origin rapidly separate from the damaged paternal chromosomes and haploid embryos develop but become lethal in a maternal effect manner (32, 33, 137). Ance encodes a putative homologue of mammalian angiotensin-converting enzyme (ACE). Compound heterozygote for two different Ance lethal alleles exhibit male sterility (34), but the molecular details are unknown. RNAi knockdown of Sf3c encoding a secreted serine protease causes male infertility (35). When a membrane serine protease encoded by ome was mutated, males became subfertile (36). RNAi knockdown of a secreted metallopeptase encoded by Mmp2 caused female subfertility because ovulation was blocked (37).

Several pest control attempts target reproduction-associated proteases. In pests Spodoptera litura and Plutella xylostella, targeted inactivation of serine protease genes Osp and Ser2, respectively, resulted in female and male infertility as also observed in silkworm moth Bombyx mori (39, 40). In other pests Hyphantria cunea, and Bactrocera dorsalis, RNAi knockdown of Hser2, and Bdcp-1 encoding serine protease and cysteine protease, respectively, also resulted in infertility (41, 42). Thus, proteases are potential targets for pest population control.

MULTICELLULAR ORGANISMS II: VERTEBRATES

Findings in vertebrates were obtained by genetic studies in rodents, fish, and human patients. Genes disrupted in these species include those encoding proteases, protease inhibitors, and non-catalytically active pseudo-proteases. Proteinolysis-related factors are included in various aspects of male and female reproductive processes such as gamete production, gamete maturation, fertilization, post-fertilization events, and mating behavior.

UPS in Gamete Production

For the fine-tuning of cellular processes, intracellular proteins are timely degraded by UPS. The proteasome localizes in the nucleus and cytoplasm where it degrades ubiquitylated proteins. Spermatoproteasome, a testis-specific proteasome, is one of the three tissue-specific proteasomes identified together with the immunoproteasome and the thymoproteasome in mammals (138). Deletion of Psme8, which encodes a testis-specific 20S proteasome component, leads to spermatogenesis arrest at the spermatocyte stage (43). Psme3 encodes REGγ, a proteasome activator. Psme3-null males are subfertile with decreased sperm number and motility (44). This is probable because REGγ regulates p53-mediated transcription of Plef, a transcription factor necessary for spermatogonial stem cell self-renewal and proliferation (139). Psme4 encodes PA200 proteasome activator. Psme4-null males have reduced fertility due to defects in meiotic spermatocytes and post-meiotic spermatids (45). Psme3;Psme4 double KO males were infertile; mutant sperm appeared morphologically normal but exhibited remarkable defects in motility and decreased proteasome activity (46).

Proteasome target proteins are ubiquitylated by E3 ubiquitin ligases which transfer the ubiquityl group from E2 ligase to the target protein. There are ~600 E3 ligases encoded in the mammalian genome (140). The ubiquitin ligases, which are not proteases but included in ubiquitin-proteasome system-mediated protein degradation, indispensable for mammalian reproduction are listed in Table 2. Here only Huwe1 is mentioned as how E3 ligases function in reproductive processes. Huwe1 ubiquitylates histone H2AX, which is phosphorylated in response to DNA damage and is essential to the efficient recognition and repair of DNA double-strand breaks. Germline-specific Huwe1 ablation increased histone H2AX level, elevated DNA damage response, and caused Sertoli cell only phenotype. Thus Huwe1 likely regulates the response to spontaneous DNA damage by UPS-mediated H2AX degradation to maintain cell survival (156).

Cullin-RING E3 ubiquitin ligases are known to be reversibly neddylated, i.e., conjugated with NEDD8, a ubiquitin-like protein. By conjugation with NEDD8, cullin-RING E3 ligases increase their stability and ligase activity. The constitutive photomorphogenic-9 signalosome (CSN) deneddylates cullin-RING E3 ligases by cleaving the isopeptide bond of neddylated lysine to regulate the cellular ubiquitlation status. COP5 is the fifth component of the CSN and abundant in mouse testis (185). Cops5-null males were infertile because of significant reduction of sperm number caused by premeiotic apoptosis of germ cells (47).

Ubiquitylated proteins can be deubiquitylated by deubiquitylating enzymes such as ubiquitin-specific proteases (USPs), cysteine endopeptidases encoded by Usp genes, thereby expression levels and activity of target proteins are regulated. USP1 deubiquitylates FANCD2 which is included in the repair of DNA crosslinks. Usp1 null males were infertile and the seminiferous tubules were markedly atrophic and mostly devoid of spermatogenic cells in the mutant testis. Usp2-null males possessed severely reduced fertility and the mutant sperm were defective in sperm motility and egg fertilizing ability in vitro (48). Germ cell-specific ablation of Usp9x using Vasa-cre possessed spermatogenic cell apoptosis at the early spermatocyte stage and resulted in complete infertility (49). Usp26 is an X-linked gene exclusively expressed in testis (186). Usp26 -null males are subfertile because of reduced number of haploid cells in testis (50, 51). Usp1-null female mice showed reduced fertility probably because of a reduced number of oocytes in ovaries (52). Thus, UPS is critically important for germ cell production in both sexes.
TABLE 2 | The ubiquitin ligases indispensable for mammalian reproduction.

| Gene Type | Gene-modified organism | Phenotype | Refs. |
|------------|------------------------|-----------|-------|
| **D. melanogaster** | | | |
| rae1 E3 ligase | component | ms (2)Z5584 mutation | Male infertility; striking defects in primary spermatocyte nuclear integrity, meiotic chromosome condensation, segregation, and spindle morphology. | (141) |
| parkin E3 ligase | element insertion | P | Female infertility. | (142) |
| cu3 E3 ligase | | E3 | Male infertility | (143) |
| C. elegans | | | |
| mel-26 E3 ligase | | EMS mutagenesis | Germ cell depletion and sterility. | (144) |
| skr-1, skr-2 E3 ligase | | RNAi component | Hermaphrodites are sterile. Arrested germline development in pachytene stage, expanded transition zone, and the presence of gaps in the gonad arm. | (145) |
| vht-1 E3 ligase | | RNAi knockdown | Reduced fertility. | (146) |
| M. musculus | | | |
| Chfr E3 ligase | KO | | Male infertility. (147) |
| Cul4a E3 ligase | KO | | Male infertility phenotype resulted from a combination of decreased spermatozoa number, excessive germ cell loss, and spermatogenic arrest, and the mutant males were infertile at 7 months old. (148) |
| Cull4b E3 ligase | Vasa-cre; Cul4b<sup>fl/fl</sup>; Cul4b<sup>-/-</sup> | Male infertility. | (149) |
| Dcaf17 E3 ligase | KO | | Male infertility. Due to abnormal sperm development. (150) |
| Dcaf8 E3 ligase | KO | | Pronounced sperm morphological abnormalities with typical bent head malformation. (151) |
| Dcun1d1 E3 ligase | KO component for neddylation | | Malformed spermatozoa with supernumerary and malpositioned centrioles. (152) |
| Fbxw7 E3 ligase | component | Amh-cre; Fbxw7<sup>fl/fl</sup> | Impaired testis development, which is characterized by age-dependent tubular atrophy, reduced sperm motility and defective acrosome formation. | (153) |
| Huwe1 E3 ligase | Ddx4-cre; Huwe1<sup>Y</sup> | Male infertility. | (154) |
| Uhrf1 E3 ligase | Str8-cre; Huwe1<sup>Y</sup> | Male infertility due to spermatogenesis arrest. Accumulation of DNA damage response protein H2AX. | (155) |
| Uhrf2 E3 ligase | Zp3-cre; Huwe1<sup>Y</sup> | Male infertility due to abnormal sperm development. | (156) |
| Uhrf3 E3 ligase | | | Pronounced spermatid abnormalities with typical bent head malformation. | (157) |
| Uhrf4 E3 ligase | | Malformed spermatozoa with supernumerary and malpositioned centrioles. | (158) |
| Uhrf5 E3 ligase | | | Female infertility. Impaired oocyte maturation, ovulation, and fertilization. | (159) |
| Uhrf6 E3 ligase | | | Female infertility. Complete lack of follicular structures resembling human premature ovarian failure. | (160) |
| Uhrf7 E3 ligase | | | Female infertility. | (161) |
| Uhrf8 E3 ligase | | | Male infertility. Degenerated testes with no organized seminiferous tubules and a complete loss of differentiated germ cells. | (162) |
| Uhrf9 E3 ligase | | | Male infertility due to impaired protamine replacement in elongated spermatids. | (163) |
| Uhrf10 E3 ligase | | | Male infertility because of arrested spermatogenesis at the pachytene stage. | (164) |
| Uhrf11 E3 ligase | | | Disrupted spermatogenesis and male infertility. | (165) |
| Uhrf12 E3 ligase | | | Male infertility. | (166) |
| Uhrf13 E3 ligase | | | Male infertility. | (167) |
| Uhrf14 E3 ligase | | | Female sterility and male infertility. Interrupted spermatogenesis because of impaired progression past meiotic metaphase I. | (168) |
| Uhrf15 E3 ligase | | | Female infertility because of impaired uterine decidualization. | (169) |
| Uhrf16 E3 ligase | | | Female infertility. Impaired oocyte maturation, ovulation, and fertilization. | (170) |
| Uhrf17 E3 ligase | | | Male infertility and female infertility. | (171) |
| Uhrf18 E3 ligase | | | Male infertility because of arrested spermatogenesis at meiotic prophase I. | (172) |
| Uhrf19 E3 ligase | | | Male infertility caused by arrested spermatogenesis at meiotic prophase I. | (173) |
| Uhrf20 E3 ligase | | | Failure of meiosis and male infertility. | (174) |
| Uhrf21 E3 ligase | | | Female infertility. | (175) |
| Uhrf22 E3 ligase | | | Male infertility because of the loss of spermatogenesis. | (176) |
| Uhrf23 E3 ligase | | | Female infertility with major defects in stability of the primordial follicle pool, ovarian folliculogenesis, ovulation and meiosis. | (177) |

(Continued)
Non-Proteasomal Intracellular and Extracellular Proteolysis Factors in Sperm Production

Intracellular and extracellular proteolysis factors critically function in spermatogenesis. Cleavage of specific peptide bonds also contributes to spermatogenesis. Apaf1 encodes a caspase activator, and Apaf1-null males are infertile because of degeneration of spermatogonia, which results in the absence of sperm (53). Agbl5 encodes an intracellular metalloprotease. Agbl5-null males are infertile because of defective spermatogenesis (54, 55). A cytosolic carboxypeptidase 1, another metalloprotease encoded by Agtpbp1 deglutamylates polyglutamylated proteins. Agtpbp1 mutant mice known as Purkinje cell degeneration (pcd) possess male infertility (109–112) because of defective spermatogenesis (110). A germ cell nuclear antigen encoded by Gcna contains a metalloprotease domain. Gcna-null males are nearly devoid of sperm and infertile (56). In human, GCNA spontaneous mutations were identified in spermatogenic failure patients (124, 125).

Separin, a caspase-like cysteine protease encoded by Esp1, plays a central role in chromosome segregation by cleaving the SCC1/RAD21 subunit of the cohesin complex (187–189). A point mutation in Esp1 which substitutes inhibitory phosphorylation site Ser1121 to Ala depletes spermatogonia because of chromosome misalignment during proliferation of the postmigratory primordial germ cells and following mitotic arrest, aneuploidy, and cell death (105). Threonine aspartase 1 (TASP1) is an intracellular endopeptidase that cleaves after distinct aspartate residues of the conserved IXQL(V)D/G motif (190). TASP1 cleaves general transcription factor TFIIIA-β to enable testis-specific transcription; Tasp1-null male mice were unable to activate spermatogenic gene activation, which lead to the release of immature germ cells and infertility (57). A serine protease ClpP is located in the mitochondrial matrix and participates in mitochondrial protein quality control by degrading misfolded or damaged proteins. In Clpp-null mutants spermatogenesis was disrupted by the spermatid stage (114). Tysnd1 encodes a serine protease that processes peroxisomal leader peptides. Tysnd1-null mutant males possess globozoospermia and their spermatozoa lack the acrosomal cap (58). Spink2 encodes a Kazal-type serine protease inhibitor abundantly expressed in testis and epididymis (191). Spink2-null males had azoospermia, and a homozygous splice mutation of SPINK2 was found in infertile men (59). Ablation of Serpina5 encoding another serine protease inhibitor also results in an abnormality in sperm production in the testis (60).

Purinomycin-sensitive aminopeptidase encoded by Npepps is also an intracellular protease. It appears to contribute indirectly to spermatogenesis. Npepps-null testes and seminal vesicles were significantly reduced in weight, spermatogenesis was impaired, and copulatory behavior was lacking. It is suggested that the defects in the testes likely arises from dysfunction of Sertoli cells, whereas the lack of copulatory behavior results from defects in the brain (115).

A null mutation of Adams2 encoding secreted metalloprotease caused male infertility (61). Decreased spermatogenesis was observed but copulatory behavior and/or copulatory plug formation may also be impaired because a copulatory plug was never observed (61).

Proteolysis Factors Associated With Sperm Function

Acrosomal Function

The acrosome is a Golgi-derived sperm head organelle in which many digestive enzymes such as proteases and hyaluronidases are included to penetrate egg surroundings. Acrosin is a serine protease and a major component of the acrosome. Although acrosin-deficient male mice are fertile (62, 63), disruption of hamster acrosin resulted in complete male infertility (120). In vitro, mutant hamster spermatozoa attached to the zona
pellucida, but failed to penetrate it (120), suggesting that acrosomal function can be attributed to specific factors in a species-specific manner.

Proprotein convertases convert inactive precursor proteins into their mature and active forms. PCSK4 is a member of proprotein convertases expressed on the sperm surface overlying the acrosome (64). Pcsk4-null males showed impaired fertility (64, 65) and mutant sperm exhibited accelerated capacitation, precocious acrosome reaction, reduced binding to egg zona pellucida (64). Acrosome formation during spermatogenesis was also abnormal (192).

Sperm Maturation
A group of genes encoding proteases, enzymatically inactive pseudoproteases, and protease inhibitors is apparently associated with the same physiological function, i.e., maturation of sperm conferring abilities to migrate into female oviduct and bind with zona pellucida. Ablation of Tmprss12 (66), Prss55 (67, 68), Tryx5 (69), Prss37 (70), Ace (71), Adam1a (72), Adam2 (73), Adam3 (74, 75), and Adam6 (76) results in deficient sperm migration into the oviduct and binding to the zona pellucida of eggs. Among them, Adam1a, Adam2, Adam3, Adam6, and Prss37 encode catalytically inactive pseudoproteases. A disintegrin and metalloproteinase domain (ADAM) 3, a catalytically inactive transmembrane pseudoprotease appears to be central to a molecular mechanism that governs sperm migratory and adhesion abilities, because ADAM3 expression is a prerequisite for sperm to acquire these abilities (193).

ADAM3 is expressed as a precursor and the processed into mature form as spermatozoa mature in epididymis (194). Similarly, enzymatically inactive pseudoproteases ADAM2 and ADAM6 are processed during sperm maturation in epididymis (195, 196). Therefore, they are rather substrates for other proteases. Ablation of ADAM2 or ADAM6 also results in significant decrease or loss of ADAM3 from epididymal sperm (74, 76) indicating the involvement of both ADAM2 and ADAM6 in ADAM3 expression. Prss37 supports ADAM3 precursor translocation to the sperm cell surface by collaborating with PDILT, a testis-specific protein disulfide isomerase indispensable for ADAM3 surface expression (197, 198). Tmprss12, Prss55, and Tryx5, all of which are serine proteases and retain catalytic triad residues, are necessary for the production or stable localization of processed ADAM3 on the cell surface of epididymal spermatozoa (66–69), although it remains uncertain whether these proteases directly cleave ADAM3.

Cystatins are secreted cysteine proteinase inhibitors. Cystatin genes Cst8, 9, 11, 12, 13, dcl1, dcl2, and l1 are clustered on mouse chromosome 2 and expressed in both testis and epididymis. Their simultaneous ablation resulted in the loss of ADAM3 from epididymal sperm and deficient sperm migration into the oviduct (77), implying the importance of regulated proteolysis in sperm maturation. Ovochymase 2 (OVCH2) is a chymotrypsin-like serine proteinase. OVCH2 is specifically expressed in the caput epididymis under the regulation of lumicrine signaling, in which testis-derived secreted protein NELL2 transiting through the luminal space acts on the epididymal epithelium by binding to its receptor ROS1 tyrosine kinase to differentiate (78). Ablation of Ovch2 results in abnormal sperm ADAM3 processing and deficient sperm migration into the oviduct (78). Thus, regulated proteolysis on or outside spermatozoa apparently modulates sperm maturation.

NL1 encoded by Mmnel1 is a zinc metallopeptidase expressed in testis. NL1 is expressed as a type II transmembrane protein but released as a soluble form. Mmnel1-null mice show normal spermatogenesis but reduced egg fertilization, suggesting the role of NL1 in sperm maturation (79). It remains, however, uncertain whether NL1 is included in ADAM3-mediated sperm maturation. Testisin encoded by Prss21 is a GPI-anchored serine protease. Prss21 KO males are subfertile because mutant spermatozoa possessed decreased motility, angulated and curled tails, and fragile necks (80). In another Prss21 mutant line in vitro sperm binding to egg zona pellucida, acrosome reaction, and fertility were decreased (81).

Other Proteolytic Factors Associated With Male Reproduction
Several cell surface and extracellular proteases and inhibitors seem to regulate male fertility in more indirect manners. Adamts16 homozygous mutant rat males resulted in cryptorchidism and male sterility (121). The mutant testis undescended during development because of the failure of gubernacular migration (122). γ-glutamyltranspeptidase 1 (GGT1) is a type II transmembrane protein which cleaves γ-glutamyl bond of extracellular glutathione (γ-Glu-Cys-Gly), glutathione conjugates, and other γ-glutamyl compounds. The resulting cysteinyl-glycine is further cleaved by dipeptidase into free amino acids. Ggt1-null males are infertile because of decreased epididymal sperm number and failure in copulatory plug formation (117). Although Ggt1-null testes was small, spermatogenesis inside seminiferous tubules appeared normal and seminal vesicles were hypoplastic. As N-acetylcysteine-fed mutant mice were fertile, the observed infertility is a consequence of cysteine deficiency (117). Carboxypeptidase E (CPE) is a metallo-carboxypeptidase and functions as a prohormone processing exopeptidase. Cpefatfat males are infertile and deficient in Pro-gonadotropin-releasing hormone processing in the hypothalamus (82). ADAM24 is a metalloproteinase localized on the mature sperm surface. Adam24-null males are subfertile and polyspermic fertilization increased in vitro and in vivo, suggesting a physiological role of ADAM24 for prevention of polyspermy (83). ADAM7 is a membrane-anchored protein with a catalytically-inactive metalloproteinase domain abundantly expressed in the epididymis (199). Adam7 ablation resulted in a modest reduction of male fertility; impaired epididymal morphology and integrity may affect sperm maturation (84).

Cystatin C encoded by Cst3 is a cysteine protease inhibitor abundantly expressed in testis and epididymis. Substitution of Leu68 to Gln is an amyloid-forming mutation found in a hereditary form of cystatin C amyloid angiopathy. Heterozygous male mice were infertile and increased levels of
amyloid was observed in the epididymal fluid (85). Nonpathological function of amyloid during epididymal sperm maturation is also suggested (200).

Immp2l encodes an inner mitochondrial membrane peptidase 2-like. Immp2l-null homozygous males were severely subfertile because of erectile dysfunction (118). Tumor necrosis factor-α (TNFα) converting enzyme encoded by Adam17 is involved in the proteolytic release of the ectodomain of diverse cell surface proteins. Conditional ablation of Adam17 with Sox9-cre severely impaired male fertility but the details are uncertain (119).

Serpin2 encodes protease nexin-1, a serine protease inhibitor expressed in seminal fluid. Serpin2-null males possessed reduced fertility because of impaired semen coagulation and copulatory plug formation (86).

Proteolytic Factors in Ovary and Follicle Development

Both intracellular and extracellular proteolytic factors are included in ovary and follicle development. Conditional ablation of separase under the control of Zp3-cre hindered extrusion of the first polar body and caused female sterility (106). Introduction of a Ser1121 to Ala deregulatory mutation into separase led to primordial germ cell apoptosis during embryonic oogenesis (107). Ablation of cytosolic carboxypeptidase 1 encoded by Agtphb1 results in female subfertility because secondary follicles poorly develop into antral follicles (113). Oocyte-specific ablation of nuclear cysteine protease separase causes female infertility because mutant oocytes are able neither to extrude polar bodies in meiosis I nor to resolve chiasmata (106).

A deregulatory mutation into separin encoded by Esp1 at early embryonic period caused primordial germ cell depletion by apoptosis during embryonic oogenesis, which led to female infertility (105, 107). The introduction of the same mutation at later oocyte development by using Zp3-cre also resulted in female infertility but because of failure in preimplantation development (108).

Matriptase encoded by Tmprss6 is a type II transmembrane serine protease which functions in iron homeostasis by cleaving cell surface proteins associated with iron absorption. Tmprss6-null females possessed marked retardation in ovarian maturation (87), probably because of severe decrease in plasma iron levels. The defective ovarian follicle development and female infertility can be mimicked by a low iron diet (201).

The inter-α-trypsin inhibitor (Iztl) family are abundantly found in body fluids including blood plasma and urine and possess inhibitory activity for serine proteases. They are composed of bikunin, a proteoglycan with a single chondroitin sulfate chain, and heavy chains covalently bound to chondroitin sulfate chain of bikunin. Iztl family members are able to transfer their heavy chains from Iztl to hyaluronan in the presence of tumor necrosis factor-stimulated gene-6. This reaction results in the modified hyaluronan covalently linked heavy chain and is necessary for hyaluronan-rich cumulus matrix expansion. When the bikunin-coding region was deleted from Ambp gene, the resulting homozygous females ovulate oocytes deficient in hyaluronan-rich cumulus matrix expansion, leading to female infertility (88, 89).

γ-secretase is an endoprotease complex that catalyzes the intramembrane cleavage of integral membrane proteins. Psen1 encodes presenillin-1, a catalytic subunit of γ-secretase. Female mice homozygous with a Leu166 to Pro mutation, an aggressive mutation found in familial Alzheimer’s disease patients, are infertile and their ovaries consisted largely of stromal elements with primordial follicles near the cortex (90).

ADAMTS1 is a secreted metalloproteinase expressed in the granulosa cell layer of mature follicles in the ovary (91). Adamts1-null females possessed lower numbers of mature follicles in the ovary and a thick and convoluted uterus (92). In another mutant mouse line, ovulation in null females was impaired because mature oocytes remained trapped in ovarian follicles (91). In zebraﬁsh, adamts9-null females possess ovarian malformation and are unable to ovulate (123).

Lomp encodes a mitochondrial serine protease. Oocyte-specific Lomp ablation by Gdf9-cre or Zp3-cre; Lompfl/fl results in female infertility because of impaired follicular development, progressive oocyte death, ovarian reserve loss (93). Furin encodes a transmembrane serine protease localized in Golgi apparatus, endosome, plasma membrane; it is necessary for mature protein release by cleaving at RX(K/R)R consensus motif. Conditional ablation of Furin by Gdf9-cre or Zp3-cre; Furinfl/fl results in female infertility because of the arrested oogenesis at early secondary follicles (94). Pappa encodes an extracellular metalloproteinase. Pappa KO females decreased their litter size and ovulatory capacity, probably because of decreased bioavailability of ovarian insulin-like growth factor (95).

Loss of GGT1 causes infertility in not only males but females. In the Ggt1-null females, antral follicles and corpora lutea were absent and follicles degenerated due to the reduced intracellular cysteine levels (117).

Mitochondrial proteases also affect ovarian follicle development. Ablation of Clpp encoding mitochondrial matrix ClpP protease caused relatively small ovaries in which follicular differentiation was impaired probably because of the reduction of the granulosa cell layers (114). When the inner mitochondrial membrane peptidase 2-like encoded by Immp2l was ablated, the resulting mutant females were deficient in folliculogenesis and ovulation and infertile, probably because of low availability of nitric oxide caused by mitochondrial dysfunction (118).

Proteolytic Factors in Post-Fertilization Events of Female Reproduction

Several proteolysis-associated secreted proteins contribute to post-fertilization events including the hardening of the egg-surrounding zona pellucida. Ovastacin encoded by Asl is a secreted metalloendopeptidase deposited in cortical granules of oocytes. Ovastatin is secreted into the extracellular space in response to egg activation triggered by fertilization. In Asl-null eggs, ZP2 cleavage necessary for zona pellucida hardening and the postfertilization block to polyspermy did not occur after fertilization (96). Fetuin is a cystatin family protease inhibitor abundantly expressed in blood plasma. Fetuin-B prevents
premature ZP hardening probably by inhibiting ovastacin derived from spontaneous cortical granule release, as fetuin-B inhibited ovastacin protease activity in vitro and Fetub-deficient oocytes undergo premature zona pellucida hardening (97).

Antithrombin encoded by Serpinc1 inhibits thrombin and some other coagulation factors by binding heparin and heparan sulfate. When an Arg48 to Cys mutation, which corresponds to human thrombosis mutation, was introduced into mice, the resulting homozygous females had decreased their litter size, probably because thrombosis occurred in placenta (98).

Adam10 encodes a membrane metalloprotease. Conditional ablation of vascular Adam10 by Tie2-Cre; Adam10fl/fl causes impaired decidualization and female subfertility (99). Adamts18 encodes a member of secreted metalloprotease ADAMTS. Adamts18-null females suffer from vaginal obstruction, due to either a dorsoventral vaginal septum or imperforate vagina and infertility or subfertility (100).

Other Proteolytic Factors in Female Reproduction

Several proteolysis-associated factors regulate female reproduction in a more indirect manner. Npepps-null females lacking a puromycin-sensitive aminopeptidase impairs corpus luteum formation and are infertile, probably because of disruption of the hypothalamic-pituitary axis (116). Plasmin is a secreted serine protease generated from plasminogen through activation by tissue-type or urokinase-type plasminogen activators. The fertility of plasmin-deficient Plg-null female mice appeared to be compromised (101, 102). It seems not to be the consequence of the impaired proteolytic process essential for ovulation, as plasminogen-deficient mice had normal ovulation efficiency (202). Timp1 encodes a tissue inhibitor of metalloproteinases 1, an inhibitor for matrix metalloproteinases. Timp1 mutation reduced the reproductive lifespan of female but not male mice (103). When Pcsk2 encoding neuroendocrine convertase 2 was ablated, the number of consecutive litters from mutant female mice was small and Pcsk2-null female mice sometimes gave birth to dead pups (104) for uncertain reason. Conditional ablation of TNFα converting enzyme by Sox9-cre; Adam17fl/fl resulted in female infertility but details are uncertain (119).

CONCLUSION AND PERSPECTIVE

By a comprehensive survey, it has been demonstrated that proteolysis regulates reproduction in various species including yeast, insects, nematodes, vertebrates, and plants. Regulation of reproduction by proteolysis already exist in unicellular yeast. In multicellular organisms, proteolysis regulates the formation and function of gametes derived from germ cells as well as the development and function of reproductive organs by somatic cells, thereby securing successful reproduction. In these cell lineages, both limited proteolysis and degradative proteolysis by ubiquitin-proteasome system play critical roles.

One of intriguing paradigms emerging in this review is that many sperm surface and extracellular proteases, pseudoproteases, and inhibitors are included in the acquisition of mammalian sperm conferring abilities to migrate into the oviduct and to bind to the zona pellucida of eggs. As spermatozoa are transcriptionally and translationally silent, post-translational modification mechanisms such as proteolysis may largely contribute to sperm maturation.

Many compounds have been designed to inhibit the enzymatic activity of proteases. Clinically, there have been numerous successes including angiotensin-converting enzyme inhibitors for cardiovascular disorders (203), thrombin inhibitors for thromboembolism and bleeding disorders (204, 205), and HIV protease inhibitors in the treatment of HIV and AIDS (206), among others (207, 208). In addition, enzymatically active proteases could also be good druggable targets for contraceptives.

Genome editing techniques developed in recent years will identify fertility-associated proteolytic factors further. In addition to identifying novel factors, more intense studies on the molecular basis of proteolysis including the identification of
substrates will clarify how proteolytic events govern reproduction. It will also clarify the physiological significance of molecular events governed by proteolysis in reproduction.

AUTHOR CONTRIBUTIONS

DK and MI wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Kahne D, Still WC. Hydrolysis of a Peptide Bond in Neutral Water. J Am Chem Soc (1988) 110(22):7529–34. doi: 10.1021/ja00230a041
2. Cherry JM, Hong EL, Amundsen C, Balakrishnan R, Binkley G, Chan ET, et al. Saccharomyces Genome Database: The Genomics Resource of Budding Yeast. Nucleic Acids Res (2012) 40(Database issue):D790–5. doi: 10.1093/nar/gks1029
3. Larkin A, Marygold SJ, Antonazzo G, Attrill H, Dos Santos G, Garapati PV, et al. FlyBase: Udates to the Drosophila Melanogaster Knowledge Base. Nucleic Acids Res (2021) 49(D1):D899–907. doi: 10.1093/nar/gkaa1026
4. Davis P, Zarowiecki M, Arnaboldi V, Becerra A, Cain S, Chan J, et al. WormBase in 2022-Data, Processes, and Tools for Analyzing Caenorhabditis Elegans. Genetics (2022) 220(4):iyac003. doi: 10.1093/genetics/iyac003
5. Puente XS, Sanchez LM, Overall CM, López-Otín C. [H]uman and Mouse Proteases: A Comparative Genomic Approach. Nat Rev Genet (2003) 4(7):544–58. doi: 10.1038/nrg1111
6. García-Lorenzo M, Sjödin A, Jansson S, Funk C. Protease Gene Families in Populus and Arabidopsis. BMC Plant Biol (2006) 6:30. doi: 10.1186/1471-2229-6-30
7. Klein T, Eckhard U, Dufour A, Solis N, Overall CM. Proteolytic Cleavage - Mechanisms, Function, and "Omic" Approaches for a Near-Ubiquitous Posttranslational Modification. Chem Rev (2018) 118(3):1137–68. doi: 10.1021/acs.chemrev.7b00120
8. Fujimura-Kamada K, Nouvet FJ, Michaelis S. A Novel Membrane-Associated Metalloprotease, Ste24p, is Required for the First Step of NH2-Terminal Processing of the Yeast a-Factor Precursor. J Cell Biol (1997) 136(2):271–85. doi: 10.1083/jcb.136.2.271
9. Adames N, Blundell K, Ashby MN, Boone C. Role of Yeast Insulin-Degrading Enzyme Homologs in Propheromone Processing and Bud Site Selection. Sci (1995) 270(5235):464–7. doi: 10.1126/science.270.5235.464
10. Hashmi S, Zhang J, Oksov Y, Ji Q, Lustigman S. The Caenorhabditis Elegans CPI-2a Cystatin-Like Inhibitor has an Essential Regulatory Role During Oogenesis and Fertilization. J Biol Chem (2006) 281(38):28415–29. doi: 10.1074/jbc.M600254200
11. Bliloch R, Kimble J. Control of Organ Shape by a Seamed Metalloprotease in the Nematode Caenorhabditis Elegans. Nat (1999) 399(6736):386–90. doi: 10.1038/112196
12. Bliloch R, Anna-Arriola SS, Gao D, Li Y, Hodgkin J, Kimble J. The Gon-1 Gene is Required for Gonadal Morphogenesis in Caenorhabditis Elegans. Dev Biol (1999) 216(1):382–93. doi: 10.1006/dbio.1999.9491
13. Kubota Y, Nishiwaki K, Ito M, Sugimoto A. The Role of Tissue Inhibitors of Metalloproteinases in Organ Development and Regulation of ADAMTS Family Metalloproteinases in Caenorhabditis Elegans. Genetics (2019) 212(2):523–35. doi: 10.1534/genetics.119.301795
14. Pispa J, Palén M, Holmberg CI, Jänö TC. Elegans Dss-1 is Functionally Conserved and Required for Oogenesis and Larval Growth. BMC Dev Biol (2008) 8:51. doi: 10.1186/1471-213X-8-51
15. Gudipati RK, Braun K, Gypas F, Hess D, Schreier J, Carl SH, et al. Protease-Mediated Processing of Argonate Proteins Controls Small RNA Association. Mol Cell (2021) 81(1):2388–2402.e8. doi: 10.1016/j.molcel.2021.03.029
16. Jarrault S, Greenwald I. Evidence for Functional Redundancy Between C. Elegans ADAM Proteins SUP-17/Kuzbanian and ADAM-4/TACE. Dev Biol (2005) 287(1):1–10. doi: 10.1016/j.ydbio.2005.08.014
17. Althoff MJ, Flick K, Trzepcz C. Collaboration Within the M1 Aminopeptidase Family Promotes Reproductive Success in Caenorhabditis Elegans. Dev Genes Evol (2014) 224(3):137–46. doi: 10.1007/s00427-014-0470-3
18. Stanfield JR, Stanfield GM. TRY-5 is a Sperm-Activating Protease in Caenorhabditis Elegans Seminal Fluid. PloS Genet (2011) 7(11):e1002375. doi: 10.1371/journal.pgen.1002375
19. Stanfield GM, Villeneuve AM. Regulation of Sperm Activation by SWM-1 is Required for Reproductive Success of C. elegans Males. Curr Biol (2006) 16(3):252–63. doi: 10.1016/j.cub.2005.12.041
20. Bhargava V, Goldstein CD, Russell L, Xu L, Ahmed M, Li W, et al. GCNA Preserves Genome Integrity and Fertility Across Species. Dev Cell (2020) 52 (1):38–52.e10. doi: 10.1016/j.devcel.2019.11.007
21. Altincicek B, Fischer M, Fischer M, Lüerssen K, Boll M, Wenzel U, et al. Role of Matrix Metalloproteinase ZMP-2 in Pathogen Resistance and Development in Caenorhabditis Elegans. Dev Comp Immunol (2010) 34(11):1160–9. doi: 10.1016/j.dci.2010.06.010
22. Adolphsen K, Amell A, Havko N, Kevorkian S, Mears K, Neher H, et al. Type-I Preynl Protease Function is Required in the Male Germline of Drosophila Melanogaster. G3 (Bethesda) (2012) 2(6):629–42. doi: 10.1534/g3.112.020188
23. Zhong L, Belote JM. The Testis-Specific Proteasome Subunit Prosalpha6T of D. Melanogaster is Required for Individualization and Nuclear Maturation During Spermatogenesis. Development (2007) 134(19):3517–25. doi: 10.1242/dev.004770
24. Koever L, Melzer J, Roca EA, Teichert D, Glatter T, Arama E, et al. The De-ubiquitylating Enzyme DUBA is Essential for Spermatogenesis in Drosophila. Cell Death Differ (2016) 23(12):19–30. doi: 10.1038/cdd.2016.79
25. Huh JR, Vernooy SY, Yu H, Yan N, Shi Y, Guo M, et al. Multiple Apoptotic Caspase Cascades are Required in Nonapoptotic Roles for Drosophila Spermatid Individualization. PloS Biol (2004) 2(1):E15. doi: 10.1371/journal.pbio.0020015
26. Tain LS, Chowdhury RB, Tao RN, Phan-Favreau H, Moisini N, Martins LM, et al. Drosophila HtrA2 is Dispersable for Apoptosis But Acts Downstream of PINK1 Independently From Parkin. Cell Death Differ (2009) 16(8):1118–25. doi: 10.1038/cdd.2009.23
27. Yun J, Cao JH, Dodson MW, Clark IE, Kapahi P, Chowdhury RB, et al. Loss-Of-Function Analysis Suggests That Omi/HtrA2 is Not an Essential Component of the PINK1/Parkin Pathway In Vivo. J Neurosci (2008) 28(53):14500–10. doi: 10.1523/JNEUROSCI.5141-08.2008
28. Laurinieycz B, Vedelek V, Kovács AL, Szalasi K, Lipinszki Z, Slezák C, et al. Sperm-Leucylaminopeptidases are Required for Male Fertility as Structural Components of Mitochondrial Paracristalline Material in Drosophila Melanogaster Sperm. *Plos Genet* (2019) 15(2):e1007987. doi: 10.1371/journal.pgen.1007987.

29. LaFlamme BA, Ram KR, Wolfner MF. The Drosophila Melanogaster Seminal Fluid Protease “Seminaise” Regulates Proteolytic and Post-Mating Reproductive Processes. *Plos Genet* (2012) 8(1):e1002435. doi: 10.1371/journal.pgen.1002435.

30. Ohsako T, Shirakami M, Oiwa K, Ibaraki K, Karr TL, Tomaru M, et al. The Drosophila Melanogaster Seminal Protease Gene, Serine Protease 2, Results in Male Sterility in Diverse Lepidopterans. *Insect Biochem Mol Biol* (2019) 116:103243. doi: 10.1016/j.ibmb.2022.103726.

31. Delabaere L, Orsi GA, Sapey-Triomphe L, Horard B, Couble P, Loppin B. The Spartan Ortholog Maternal Haploid Is Required for Paternal Chromosome Germ Cell Survival and Reproduction Revealed Rapid Evolution of Phenotypic Effects at Adult Stages. *Fly (Austin)* (2011) 5(4):345–51. doi: 10.4161/fly.5.4.17808.

32. Hichra CI, Song C, LaMonte G, Fetalvero K, Hinchman K, Phan H, et al. Identification and Partial Characterization of the Enzyme of Omega: One of Five Putative DPP IV Genes in Drosophila melanogaster. *J Insect Sci* (2005) 5:262. doi: 10.1093/jis/5.1.26.

33. Tatei K, Cai H, Ip YT, Levine M. Race: A Drosophila Homologue of the Protein That Mediates a Postmating, Prezygotic Phenotype in a Cricket. *Mech Dev* (1995) 51(2–3):157–68. doi: 10.1016/0925-4773(95)00349-5.

34. Yeung JY, Naus TA, Shi J, Tadros H, van de Loo J-W, et al. Sperm From Mice Genetically Deficient in Apaf-1 Defective Mice Exhibit Male Infertility. *Dev Biol* (2000) 218(2):248–58. doi: 10.1006/dbio.2000.9834.

35. Chen S, Yang H, Krinsky BH, Zhang A, Long M. Roles of Young Serine-Endopeptidase Genes in Survival and Reproduction Revealed Rapid Evolution of Phenotypic Effects at Adult Stages. *Fly (Austin)* (2011) 5(4):345–51. doi: 10.4161/fly.5.4.17808.

36. Shi J, Tadros H, van de Loo J-W, et al. Sperm From Mice Genetically Deficient in the PCSK4 Proteinase Exhibit Accelerated Capacitation, With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

37. Kishi K, Uchida A, Takase HM, Suzuki K, Hurok II, Mtsukawa N, et al. Spermatoogenic Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

38. Bedard N, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, et al. Mice Lacking the USP2 Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

39. Behin Y, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, et al. Mice Lacking the USP2 Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

40. Behin Y, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, et al. Mice Lacking the USP2 Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

41. Behin Y, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, et al. Mice Lacking the USP2 Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

42. Behin Y, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, et al. Mice Lacking the USP2 Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.

43. Behin Y, Yang Y, Gregory M, Cyr DG, Suzuki J, Yu X, et al. Mice Lacking the USP2 Deubiquitinating Enzyme Have Severe Male Subfertility Associated With Defects in Fertilization and Sperm Motility. *Bio Reprod* (2011) 85(3):594–604. doi: 10.1095/biolreprod.110.088542.
76. Voronina VA, Harris FM, Schmahl J, Galligan C, Oristian D, Zammara C, Shen C, Kuang Y, Liu J, Feng J, Chen X, Wu W, et al. Prss37 is Required for Fertility. *Biol Reprod* (2020) 103(2):254–63. doi: 10.1093/bioreprod/oa0060

77. Shang X, Chen C, Liu J, Tang L, Zhang H, Wang Y, et al. Serine Protease PRSS55 is Crucial for Male Fertility via Affecting Sperm Migration and Sperm-Egg Binding. *Cell Mol Life Sci* (2018) 75(23):4371–84. doi: 10.1007/s00018-018-2878-9

78. Kobayashi K, Endo T, Matsumura T, Lu Y, Yu Z, Matzuw MM, et al. Prss55 But Not Prss51 is Required for Male Fertility in Mice. *Biol Reprod* (2020) 103(2):232–34. doi: 10.1093/bioreprod/aao401

79. Zhang H, Li Y, Cui K, Chen X, Min W, et al. Male Fertility in Mus Musculus Requires the Activity of TRYX5 in Sperm Migration Into the Oviduct. *J Cell Physiol* (2020) 255(9):6058–72. doi: 10.1002/jc.29534

80. Shen C, Kuang Y, Liu J, Feng J, Chen X, Wu W, et al. Prss37 is Required for Male Fertility in the Mouse. *Biol Reprod* (2013) 88(5):123–11. doi: 10.1095/bioreprod.110.107208

81. Esther CR, Howard TE, Marino EM, Goddard JM, Capecchi MR, Bernstein CR, Howard TE, Marino EM, Goddard JM, Capecchi MR, Bernstein CR, Yue H, et al. Deletion of Adam6 in Mus Musculus Leads to Male Subfertility and Compromises Mouse Ovarian Steroidogenesis and Female Fertility. A Study by Inactivation of the Bikunin Gene in Mice. *J Biol Chem* (2001) 274(13):11545–60. doi: 10.1074/jbc.M100303200

82. Zhuo L, Yoneda M, Zhao M, Yingsung W, Yoshida N, Kitagawa Y, et al. Defect in SHAP-Hyaluronan Complex Causes Severe Female Infertility. A Study by Inactivation of the Bikunin Gene in Mice. *J Biol Chem* (2001) 276(17):1154–60. doi: 10.1074/jbc.M000992200

83. Vidal R, Sammeta N, Garringer HJ, Sambamurti K, Miravalle L, Lamb BT, et al. The Psen1-I166P Knock-in Mutation Leads to Amyloid Deposition in Human Wild-Type Amyloid Precursor Protein YAC Transgenic Mice. *FEBS Lett* (2017) 587(26):2899–910. doi: 10.1002/elsb2.201505452

84. Mizutani T, Russell DL, Wilson T, Brasted M, Tkalcevic J, Salamonsen LA, et al. Adamts-1 is Essential for the Development and Function of the Urogenital System. *Biol Reprod* (2004) 70(4):1096–105. doi: 10.1095/biolreprod.103.023911

85. Shindo T, Kurihara H, Kuno K, Yokoyama H, Wada T, Kurihara Y, et al. ADAMTS-1: A Metalloproteinase-Disintegrin Essential for Normal Growth, Fertility, and Organ Morphology and Function. *J Clin Invest* (2000) 105(10):1345–52. doi: 10.1172/JCI13655

86. Shindo T, Kurihara H, Kuno K, Yokoyama H, Wada T, Kurihara Y, et al. ADAMTS-1: A Metalloproteinase-Disintegrin Essential for Normal Growth, Fertility, and Organ Morphology and Function. *J Clin Invest* (2000) 105(10):1345–52. doi: 10.1172/JCI13655

87. Mittaz L, Russell DL, Wilson T, Brasted M, Tkalcevic J, Salamonsen LA, et al. Adamts-1 is Essential for the Development and Function of the Urogenital System. *Biol Reprod* (2004) 70(4):1096–105. doi: 10.1095/biolreprod.103.023911

88. Zhuo L, Yoneda M, Zhao M, Yingsung W, Yoshida N, Kitagawa Y, et al. ADAMTS-1: A Metalloproteinase-Disintegrin Essential for Normal Growth, Fertility, and Organ Morphology and Function. *J Clin Invest* (2000) 105(10):1345–52. doi: 10.1172/JCI13655

89. Vidal R, Sammeta N, Garringer HJ, Sambamurti K, Miravalle L, Lamb BT, et al. The Psen1-I166P Knock-in Mutation Leads to Amyloid Deposition in Human Wild-Type Amyloid Precursor Protein YAC Transgenic Mice. *FEBS Lett* (2017) 587(26):2899–910. doi: 10.1002/elsb2.201505452

90. Vidal R, Sammeta N, Garringer HJ, Sambamurti K, Miravalle L, Lamb BT, et al. The Psen1-I166P Knock-in Mutation Leads to Amyloid Deposition in Human Wild-Type Amyloid Precursor Protein YAC Transgenic Mice. *FEBS Lett* (2017) 587(26):2899–910. doi: 10.1002/elsb2.201505452

91. Mittaz L, Russell DL, Wilson T, Brasted M, Tkalcevic J, Salamonsen LA, et al. Adamts-1 is Essential for the Development and Function of the Urogenital System. *Biol Reprod* (2004) 70(4):1096–105. doi: 10.1095/biolreprod.103.023911
102. Lund LR, Bjorn SF, Sternlicht MD, Nielsen BS, Solberg H, Usher PA, et al. Lactational Competence and Involution of the Mouse Mammary Gland Requires Plasminogen. Development (2000) 127(20):4841–92. doi: 10.1242/dev.127.20.4841

103. Nothnick WB. Reduction in Reproductive Lifespan of Tissue Inhibitor of Metalloproteinase 1 (TIMP-1)-Deficient Female Mice. Reproduction (2001) 122(6):923–7. doi: 10.1530/rep.12220923

104. Furuta M, Yano H, Zhou A, Rouille

105. Kudo NR, Wassmann K, Anger M, Schuh M, Wirth KG, Xu H, et al. Mutation Leads to Genome Instability and Primordial Germ Cell Depletion During Oogenesis. PloS One (2011) 6(4):e18763. doi: 10.1371/journal.pone.0018763

106. Huang X, Andreu-Vieyra CV, York JP, Hatcher R, Lu T, Matzuk MM, et al. Inhibitory Phosphorylation of Separse is Essential for Genome Stability and Viability of Mouse Embryonic Germ Cells. PloS Biol (2008) 6(1):e15. doi: 10.1371/journal.pbio.0060015

107. Xu J, Wang M, Gao X, Hu B, Du Y, Zhou J, et al. Separse Phosphosulfate Mutation Leads to Genome Instability and Primordial Germ Cell Depletion During Oogenesis. PloS One (2011) 6(4):e18763. doi: 10.1371/journal.pone.0018763

108. Han X, Andreu-Vieyra CV, Wang M, Cooney AJ, Matzuk MM, Zhang P. Requirement of mouse embryos depends on inhibitory phosphorylation of Separse to Prevent Chromosome Missegregation. Mol Cell Biol (2009) 29(6):1498–505. doi: 10.1128/MCB.01778-08

109. Mullen RJ, Eicher EM, Sidman RL. Purkinje Cell Degeneration, a New Neurological Mutation in the Mouse. Proc Natl Acad Sci (1976) 73(1):208–12. doi: 10.1073/pnas.73.1.208

110. Krulewski TF, Neumann PE, Gordon JW. Insertional Mutation in a Cytosolic Carboxypeptidase 1 Leads to Subfertility Due to the Reduced Accumulation of CLPX, mtDNA and Neprilysins: An Evolutionarily Conserved Family of Metalloproteases That Are Associated With Human Male Infertility. Eur J Hum Genet (2021) 29(12):1781–8. doi: 10.1038/s41431-021-00946-2

111. Hardy JJ, Wyrwoll MJ, Mcfadden W, Malcher A, Rotte N, Pollock NC, et al. Variants in GCNA, X-Linked Germ-Cell Genome Integrity Gene, Identified in Men With Primary Spermatogenic Failure. Hum Genet (2021) 140(8):1169–82. doi: 10.1007/s00439-021-02287-y

112. Gao H, Zhang Y, Wang W, Zhao K, Liu C, Bai I, et al. Two Membrane-Anchored Aspartic Proteases Contribute to Pollen and Ovule Development. Plant Physiol (2017) 175(1):219–39. doi: 10.1104/pp.16.01719

113. Ge X, Dietrich C, Matsuno M, Li G, Berg H, Xia Y. An Arabidopsis Aspartic Protease Functions as an Anti-Cel-Death Component in Reproduction and Embryogenesis. EMBO Rep (2005) 6(3):282–8. doi: 10.1038/sj.emb.7400357

114. Phan HA, Iacoume S, Li SF, Parish RW. The MYB80 Transcription Factor is Required for Pollen Development and the Regulation of Tapetal Programmed Cell Death in Arabidopsis Thaliana. Plant Cell (2011) 23(6):2209–24. doi: 10.1105/tpc.110.082651

115. Zhang D, Liu D, Lv X, Wang Y, Xun L, Liu Z, et al. The Cysteine Protease CEP1, A Key Executor Involved in Tapetal Programmed Cell Death, Regulates Pollen Development in Arabidopsis. Plant Cell (2014) 125(7):239–61. doi: 10.1105/tpc.113.127282

116. Liu L, Jiang Y, Zhang X, Wang X, Wang Y, Han Y, et al. Two SUMO Proteases SUMO PROTEASE RELATED TO FERTILITY1 and 2 Are Required for Fertility in Arabidopsis. Plant Physiol (2017) 175(4):1703–19. doi: 10.1104/pp.17.00021

117. Huang J, Zhao X, Cheng K, Jiang Y, Ouyang Y, Xu C, et al. OsaAP65, A Rice Aspartic Protease, is Essential For Male Fertility and Plays a Role In Pollen Germination and Pollen Tube Growth. J Exp Bot (2013) 64(11):3531–60. doi: 10.1093/jxb/ert173

118. Thaler CD, Miyata H, Haimo LT, Cardullo RA. Waveform Generation Is Controlled by Phosphorylation and Swimming Direction Is Controlled by Ca2+ in Sperm From the Mosquito Culex Quinquefasciatus. Biol Reprod (2013) 89(6):135. doi: 10.1093/biolreprod.113.109488

119. Miyata H, Thaler CD, Haimo LT, Cardullo RA. Protease Activation and the Signal Transduction Pathway Regulating Motility in Sperm From the Water Strider Aquarius Remigis. Cytoskeleton (2012) 69(4):207–20. doi: 10.1002/cm.21012

120. Gosney R, Liu W-S, Lamunyon CW. A Novel Function for the Presenilin Family Member Spe-4: Inhibition of Spermatal Activation in Caenorhabditis Elegans. BMC Dev Biol (2008) 8:84. doi: 10.1186/1471-213X-8-44

121. Rodriguez A, Oliver H, Zou H, Chen P, Wang X, Abrams JM. Dark is a Drosophila Homologue of Apaf-1/CED-4 and Functions in an Evolutionarily Conserved Death Pathway. Nat Cell Biol (1999) 1(5):272–9. doi: 10.1038/ncb7184

122. Sitnik JL, Francis C, Hens K, Huybrechts R, Wolfner MF, Callaerts P. NEPRILYSINS: An Evolutionarily Conserved Family of Metalloproteases That Play Important Roles in Reproduction in Drosophila. Genes (2014) 196(3):781–9. doi: 10.1534/genes.113.160945

123. Tang X, Cao J, Zhang L, Huang Y, Zhang Q, Rong YS. Maternal Haploid, A Metalloprotease Enriched at the Largest Satellite Repeat and Essential for Genome Integrity in Drosophila Embryos. Genes (2017) 206(4):1829–39. doi: 10.1534/genes.117.002699
138. Qian M-X, Pang Y, Liu CH, Haratake K, Du B-Y, Ji D-Y, et al. Acetylation-Mediated Proteasomal Degradation of Core Histones During DNA Repair and Spermatogenesis. Cell (2013) 153(5):1012–24. doi: 10.1016/j.cell.2013.04.032

139. Costoya JA, Hobbs RM, Barma M, Cattoretti G, Manova K, Sukhwani M, et al. Essential Role of Paf6 in Maintenance of Spermatogonial Stem Cells. Nat Genet (2004) 36(6):653–9. doi: 10.1038/ng1367

140. Jeffry P, Haakonsen DL, Rapé M. An E3 Ligase Guide to the Galaxy of Small-Molecule-Induced Protein Degradation. Chem Chem Biol (2021) 28(7):1000–13. doi: 10.1016/j.chembiol.2021.04.002

141. Volpi S, Bongiorni S, Fabbretti F, Wakimoto BT, Panterra G. Drosophila Rac1 is Required for Male Meiosis and Spermatogenesis. J Cell Sci (2013) 126 (Pt 16):3541–51. doi: 10.1242/jcs.111328

142. Ottone C, Galasso A, Gemi M, Pisa V, Gigliotti S, Pizziconi F, et al. Diminution of Eif6e Activity Suppresses Parkin Mutant Phenotypes. Gene (2011) 470(1-2):12–9. doi: 10.1016/j.gene.2010.09.003

143. Arama E, Bader M, Riekhof GE, Steller H. A Ubiquitin Ligase Complex Regulates Caspase Activation During Sperm Differentiation in Drosophila. PLoS Biol (2007) 5(10):e251. doi: 10.1371/journal.pbio.0050251

144. Luke-Glaser S, Pintard L, Tyers M, Peter M. The AAA-ATPase FIGL-1 Controls Mitotic Progression, and its Levels are Regulated by the CUL-3mel-Linked Huwe1 Is Essential for Spermatogenesis and Male Fertility. Dev Biol (2011) 354(1–2):92–100. doi: 10.1016/j.ydbio.2011.05.661

145. Zhang H, Chen F, Dong H, Xie M, Zhang H, Chen Y, et al. Loss of Fbxw7 in Mice Apoptosis of Sertoli Cells After Conditional Ablation of Murine Double Minute 2 (Mdm2) Gene is F53-Dependent and Results in Male Sterility. Cell Death Differ (2016) 23(3):521–30. doi: 10.1038/cdd.2015.120

146. Cheng D, Xiong C, Liu J, Cui S, Wang S, Li H, et al. The Effect of Mahogonin Gene Mutant on Reproduction in Male Mice: A New Sight for Infertility? Andrologia (2014) 46(2):98–105. doi: 10.1111/and.12050

147. Wang X, Kang J-Y, Wei L, Yang X, Sun H, Yang S, et al. PHF7 is a Novel Histone H2A E3 Ligase Prior to Histone-to-Protamine Exchange During Spermatogenesis. Development (2019) 146(13):dev175547. doi: 10.1242/dev.175547

148. Xia Z, Song Z, Li G, Tu H, Liu W, Liu Y, et al. H2B Ubiquitination Regulates Meiotic Recombination by Promoting Chromatin Relaxation. Nuclear Acids Res (2016) 44(20):9681–97. doi: 10.1093/nar/gkw652

149. Melnick AF, Gao Y, Liu J, Deng D, Predom A, Kelly C, et al. RNF216 is Essential for Spermatogenesis and Male Fertility. J Biol Reprod (2019) 100 (5):1132–4. doi: 10.1093/biore/oio006

150. Guo Y, Song Y, Guo Z, Hu M, Liu B, Duan H, et al. Function of RAD6B and RNFLF in Spermatogenesis. Cell Cycle (2018) 17(2):162–73. doi: 10.1080/15384101.2017.1361066

151. Lu L-Y, Wu J, Ye L, Gavrila GB, Saunders TL, Yu X. RNFLF-Dependent Histone Modifications Regulate Nucleosome Removal During Spermatogenesis. Dev Cell (2010) 18(3):371–84. doi: 10.1016/j.devcel.2010.01.010

152. Dickins RA, Frew IJ, House CM, O’Bryan MK, Holloway AJ, Havir I, et al. The Ubiquitin Ligase Component Siah1a Is Required for Completion of Meiosis I in Male Mice. Mol Cell Biol (2002) 22(7):2294–303. doi: 10.1128/MCB.22.7.2294-2303.2002

153. Hai L, Szwarc MM, He B, Lonard DM, Kommagani R, DeMayo FJ, et al. Uterine Function in the Mouse Requires Speckle-Type Poz Protein. Dev Biol (2019) 438(2):229–42. doi: 10.1016/j.ydbio.2019.08.006

154. Wei J, Chen L, Li F, Yuan Y, Wang Y, Xia W, et al. HRD1-ERAD Controls Production of the Hepatokine FGF21 Through CREGB Polyubiquitination. EMBO J (2017) 36(22):28992–4. doi: 10.15252/embj.201828992

155. Kettunen KM, Karikoski R, Hamaläinen RH, Toivonen TT, Antonenkov VD, Kuleskaya N, et al. Trim37-Deficient Mice Recapitulate Several Features of the Multi-Organ Disorder Multilayer Nanism. Biol Open (2016) 5(5):584–95. doi: 10.1242/bio.016246

156. Torres-Fernández LA, Emich J, Port Y, Mitschka S, Wöste M, Schneider S, et al. TRIM71 Deficiency Causes Germ Cell Loss During Mouse Embryogenesis and Is Associated With Human Male Infertility. Front Cell Dev Biol (2021) 9(6):856–69. doi: 10.3389/fcell.2021.653896

157. Kwon YT, Xia Z, An JY, Tasaki T, Davydov IV, Seo JW, et al. Female Lethality and Apoptosis of Spermatocytes in Mice Lacking the UBR2 Ubiquitin Ligase of the N-End Rule Pathway. Mol Cell Biol (2003) 23 (22):8255–71. doi: 10.1128/MCB.23.22.8255-8271.2003

158. Han J, Jiang N, Sun S, Jiang H, Xu J, Jiang X, et al. UHRF1-Repessed 5-Hydroxymethylcytosine is Essential for the Male Meiotic Prophase I. Cell Death Dis (2020) 11(2):142. doi: 10.1038/s41419-020-2333-3

159. Cao Y, Li M, Liu F, Ni X, Wang S, Zhang H, et al. Deletion of Maternal UHRF1 Severely Reduces Mouse Oocyte Quality and Causes Developmental Defects in Preimplantation Embryos. FASEB J (2019) 33(7):8294–305. doi: 10.1096/fj.201801698RBB

160. Rodríguez A, Briley SM, Patton BK, Tripurani SK, Rajapakse K, Coarfa C, et al. Loss of the E2 SUMO-Conjugating Enzyme Ube2i in Oocytes During
Ovarian Folliculogenesis Causes infertility in mice. Development (2019) 146 (23):dev176701. doi: 10.1242/dev.176701

Koenig PA, Nicholls PK, Schmidt FF, Hagwara M, Murayama T, Frydman GH, et al. The E2 ubiquitin-conjugating enzyme UBE2J1 is required for Spermiogenesis in mice. J Biol Chem (2014) 289(50):34490–502. doi: 10.1074/jbc.M114.604132

Grzmił P, Altman ME, Adham IM, Engel U, Jeng J, Mariant A, et al. Embryo Implantation Failure and Other Reproductive Defects in Ube2z1-Deficient Mice. Reproduction (2013) 145(4):45–56. doi: 10.1530/REP-12-0054

Jiang X, Wang X, Zhang X, Xiao Z, Zhang C, Liu X, et al. A Homozygous RNF220 Mutation Leads to Male Infertility With Small-Headed Sperm. Gene (2019) 688:13–8. doi: 10.1016/j.gene.2018.11.074

Wang H, Lu Y, Jiang T, Berg H, Li C, Xia Y. The Arabidopsis U-Box/ARM Repeat E3 Ligase ATuP48 Influences Growth and Degeneration of Tapetal Cells, and its Mutation Leads to Conditional Male Sterility. Plant J (2013) 74 (3):511–23. doi: 10.1111/tpj.12146

Byzova MV, Franken J, Aarts MG, de Almeida-Engler J, Engler G, Mariani C, et al. Arabidopsis STERILE APETALA1, a Multifunctional Gene Regulating Inflorescence, Flower, and Ovule Development. Genes (2019) 13 (8):1002–14. doi: 10.1007/s12228-018-01002-2

Ling Y, Zhang C, Chen T, Hao H, Liu P, Bressan RA, et al. Mutation in SUMO E3 Ligase, SIZ1, Disrupts the Mature Female Gametophyte in Arabidopsis. Plant Cell. Protoc. One (2012) 7(1):e29470. doi: 10.1371/journal.pone.0029470

Liu M, Shi S, Zhang S, Xu P, Liu J, Liu Y, et al. SUMO E3 Ligase AtMMSS1 is Required for Normal Meiosis and Gametophyte development in Arabidopsis. BMC Plant Biol (2014) 14:153. doi: 10.1186/1471-2229-14-153

Thangasamy S, Guo C-L, Chuang M-H, Lai M-H, Chen J, Iauh G-Y. Rice SIZ1, a SUMO E3 Ligase, Controls Spikelet Fertility Through Regulation of Anthel Dehisce. New Phycol (2011) 189(3):869–82. doi: 10.1111/j.1469-8137.2010.03588.x

Cayli S, Ozcukl S, Erdemir F, Tas U, Aslan H, Yener T, et al. Developmental Expression of P97/VCPC (Valosin-Containing Protein) and Jabi/CSN5 in the Rat Testis and Epididymis. Reprod Biol Endocrinol (2011) 9:117. doi: 10.1186/1477-7827-9-117

Wang PJ, McCarrney JR, Yang F, Page DC. An Abundance of X-Linked Genes and Fertility in Mice Carrying a Mutation in the Spink2 Gene Expressing a Pharmacologic Inhibitor of the Protease Taspase1 Effectively Inhibits Cancer Res (2012) 72(3):736–43. doi: 10.1016/j.jbc.2011.12.021

Tuohi K, Ikawa M, Benham AM, Okabe M. Protein Disulfide Isomerase Homolog PDH1 is Required for Quality Control of Sperm Membrane Protein ADAM3 and Male Fertility. PNAS (2012) 109(10):3850–5. doi: 10.1073/pnas.1117963109

Xiong W, Shen C, Li C, Zhang X, Ge H, Tang L, et al. Dissecting the PRSS37 Interacome and Potential Mechanisms Leading to ADAM3 Loss in PRSS37Null Sperm. J Cell Sci (2021) 134(10):jcs258426. doi: 10.1242/jcs.258426

Cornwall GA, Hisa N. ADAM7, A Member of the ADAM (a Disintegrin and Metalloproteinase) Gene Family is Specifically Expressed in the Mouse Anterior Pituitary and Epididymis. Endocrinol (1997) 138(10):4262–72. doi: 10.1210/endo.138.10.53468

Whelly S, Johnson S, Powell J, Borchardt C, Hastert MC, Cornwall GA. Nonpathological Extracellular Amyloid is Present During Normal Epididymal Sperm Maturation. PloS One (2012) 7(5):e36394. doi: 10.1371/journal.pone.0036394

Tonai S, Kawabata A, Nakanishi T, Lee JY, Okamoto A, Shimada M, et al. Iron Deficiency Induces Female Infertility in Order to Failure of Follicular Development in Mice. J Reprod Dev (2020) 66(5):475–83. doi: 10.1262/jrd.2020-074

Ny A, Leonardsson G, Haggglund AC, Haggflod P, Ploplis VA, Carmeliet P, et al. Ovulation in Plasminogen-Deficient Mice. Endocrinol (1999) 140 (11):5030–5. doi: 10.1210/endo.140.11.7113

Piepho RW. Overview of the angiotensin-converting-enzyme inhibitors. Am J Health Syst Pharm (2000) 57(Suppl 1):S3–7. doi: 10.1093/jjhp/57.suppl_1.S3

Straub A, Roehrig S, Hillisch A. Oral, Direct Thrombin and Factor X Inhibitors: The Replacement for Warfarin, Leeches, and Pig Intestines? Angew Chem Int Ed Engl (2011) 50(20):4574–90. doi: 10.1002/anie.201004575

Nutescu EA, Wittkowsky AK. Direct Thrombin Inhibitors for Anticoagulation. Ann Pharmacother (2004) 38(11):99–109. doi: 10.1345/aph.1D066

Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 Protease Inhibitors. A Rev Clin JAMA (1997) 277(2):145–53. doi: 10.1001/jama.1997.03540260500357

Agborewo AA, Huston WM, Gamble AB, Tyndall JDA. Proteases and Protease Inhibitors in Infectious Diseases. Med Res Rev (2018) 38(4):1295–331. doi: 10.1002/med.21475

Eatemadi A, Aiyelabegun HT, Negahdari B, Mazlomi MA, Daraee H, Daraee N, et al. Role of Protease and Protease Inhibitors in Cancer Pathogenesis and Treatment. BioMed Pharmacother (2017) 86:221–31. doi: 10.1016/j.biopha.2016.12.021

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