Reduced Fertility and Altered Epididymal and Sperm Integrity in Mice Lacking ADAM7

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ABSTRACT

The mammalian epididymis is a highly convoluted tubule that connects the testis to the vas deferens. Its proper functions in sperm transport, storage, and maturation are essential for male reproduction. One of the genes predominately expressed in the epididymis is ADAM7 (a disintegrin and metalloprotease 7). Previous studies have shown that ADAM7 synthesized in the epididymis is secreted into the epididymal lumen and is then transferred to sperm membranes, where it forms a chaperone complex that is potentially involved in sperm fertility. In this study, we generated and analyzed mice with a targeted ADAM7-null mutation. We found that the fertility of male mice was modestly but significantly reduced by knockout of Adam7. Histological analyses revealed that the cell heights of the epithelium were dramatically decreased in the caput of the epididymis of Adam7-null mice, suggesting a requirement for ADAM7 in maintaining the integrity of the epididymal epithelium. We found that sperm from Adam7-null mice exhibit decreased motility, tail deformation, and altered tyrosine phosphorylation, indicating that the absence of ADAM7 leads to abnormal sperm functions and morphology. Western blot analyses revealed reduced levels of integral membrane protein 2B (ITM2B) and ADAM2 in sperm from Adam7-null mice, suggesting a requirement for ADAM7 in normal expression of sperm membrane proteins involved in sperm functions. Collectively, our study demonstrates for the first time that ADAM7 is required for normal fertility and is important for the maintenance of epididymal integrity and for sperm morphology, motility, and membrane proteins.

ADAM, epididymis, fertility, gene knockout, male infertility, sperm, sperm maturation

INTRODUCTION

The epididymis, a tightly coiled tubule that connects the efferent duct at the rear of the testis to the vas deferens, can be divided into three main regions: the caput (head), corpus, and cauda. The adult epididymal epithelium consists of several cell types, including principal, basal, clear, narrow, apical, and halo cells [1]. The principal cells form the majority of the epithelium and have absorptive and secretory functions. Sperm leaving the testis pass through the efferent ducts and enter into the epididymis, where they acquire fertilizing ability. During epididymal transit, sperm are mixed with and exposed to intraluminal fluid. The epididymal fluid contains electrolytes whose ionic composition is regulated by transepithelial water reabsorption throughout the epididymal tubule [2]. The epididymal fluid also contains several hundred proteins, most of which are actively secreted by the epididymal epithelium. The secreted proteins interact with sperm, modifying sperm surface proteins or becoming incorporated into sperm subcellular domains [3, 4]. Mutation of genes encoding secreted proteins often leads to male infertility as a result of defective sperm motility or morphology [3, 5–10]. In addition to merocrine secretion, the common mode of secretion, epithelial cells of the epididymis secrete through an additional pathway referred to as apocrine secretion. In this latter process, the apical region of the epididymal principal cells emits protrusions or blebs, forming vesicular structures called epididymosomes [4].

ADAMs (a disintegrin and metalloprotease) are a family of membrane-anchored proteins whose members are widely distributed in different species and are present in a variety of tissues. At least 34 and 26 ADAM family genes have been identified in mice and humans, respectively [11]. One ADAM gene, ADAM7, is expressed predominantly in the epididymis [12–14]. The ADAM7 protein is localized to the tubules of all epididymal regions, including the proximal caput, corpus, and cauda [15]. It has been found that ADAM7 synthesized in the epididymis is secreted and transferred to mature sperm in mice and humans [13, 15, 16]. Our previous investigation of mouse ADAM7 demonstrated a unique mode of secretion and interaction of the protein during the epididymis-to-sperm transfer process; ADAM7 secreted into the epididymal lumen is associated with epididymosomes, and relocation of the protein to sperm occurs without processing of the protein [17]. We also previously found that mouse ADAM7 is associated with calnexin (CANX), heat shock protein 5 (HSPA5), and integral membrane protein 2B (ITM2B) in sperm membranes [18]. In particular, we found that the association of ADAM7 with ITM2B is promoted during sperm capacitation because of a conformational change in ADAM7 that occurs in concert with the capacitation process [18]. Finally, more than half of ADAM7 transferred from the epididymis was found to be lost in sperm lacking ADAM2 or...
ADAM3, suggesting the involvement of ADAM7 in ADAM2- and ADAM3-dependent association with sperm during epididymal transit [19]. ADAM2 and ADAM3 are known to be critical for sperm migratory and fertilization functions [11].

In this study, we generated mice with a targeted disruption in the Adam7 gene and examined the phenotypes of the knockout mice. We found that Adam7-null mice have a reduction in fertility and exhibit multiple defects in male reproduction. The Adam7+/− epididymis shows an altered structure, characterized by a decreased thickness of the caput epithelium. Sperm from Adam7-null mice are abnormal in motility, flagellum morphology, and tyrosine phosphorylation pattern. In addition, the expression levels of surface proteins known to be associated with or related to ADAM7 are decreased in the mutant sperm. The present study is the first to report the in vivo functions of ADAM7 in male reproduction.

MATERIALS AND METHODS

Generation of Adam7+/− Mice and Genotyping

Adam7+/− heterozygous mice were commercially generated by the Texas Institute for Genomic Medicine. Chimeric mice were mated with C57BL/6 females, and germ line transmission in pups was confirmed by Southern blot analysis [20, 21]. The Adam7+/− line was obtained by crossing heterozygotes. Mice were genotyped by polymerase chain reaction (PCR) using genomic DNA from tail samples and the following primers: wild-type (WT)-specific reverse, 5′-ATTTGATCTAAGGAAAGTTGGC-3′; mutation-specific reverse, 5′-CCCTAGGAATGCTCGTCAGA-3′; and common forward, 5′-GGACAAATGTTGGAATACGTC-3′. The thermocycling conditions were used 30 cycles of 94°C for 30 sec (denaturation), 54°C for 30 sec (annealing), and 72°C for 30 sec (extension). All animal investigations were carried out according to the guidelines of the Animal Care and Use Committee of Gwangju Institute of Science and Technology (GIST). The protocol was approved by the Animal Care and Use Committee of GIST (permit no. GIST 2011-13).

Fertility Test

The fertility of Adam7+/− males was tested by placing individual Adam7+/− or WT males (8-wk-old) with two C57BL/6 females for 2 wk to 6 mo. The females were checked for the presence of vaginal plugs and pregnancy. The number of pups was counted, and fertility rate was calculated.

In Vivo Fertilization Assay

The fertility of Adam7+/− males was further examined using superovulated C57BL/6 WT females. Females (8–9 wk old) were induced to ovulate using intraperitoneal injection of equine chorionic gonadotropin (eCG; Sigma) on Day 1 and human chorionic gonadotropin (hCG; Sigma) on Day 3 of treatment. The Adam7+/− and Adam7−/− mice were mated with gonadotropin-injected females (one male per two females). After formation of vaginal plugs, fertilized oocytes were obtained from ampullae and cultured in potassium-supplemented simplex optimized medium (KSOM; lab made) for 24 h at 37°C in a 5% CO2 environment. Fertility efficiency was evaluated by counting the number of two-cell-stage cells relative to the total number at 24 h.

Histological Analysis

The epididymides were dissected and fixed overnight by submersion in Bouin solution (Sigma). Tissue was dehydrated, paraffin embedded, and sectioned (5 μm thick) using a Microm microtome. Sections of WT and Adam7-null tissue were stained with hematoxylin and eosin (H&E; Sigma).

Sperm Analysis

Sperm from the cauda epididymis, vas deferens, and uterus were collected. Sperm were counted in a hemocytometer under a light microscope. Sperm motility was assessed by diluting a drop of sperm-containing solution in warm medium or PBS, followed by immediate observation of the diluted samples using an inverted microscope (Leica Microsystems). Sperm with movement, regardless of high or low motility, were considered as motile sperm. At least 10 widely spaced fields were examined. Sperm were further observed for morphological changes under a light microscope (Leica Microsystems). For Western blot analysis, sperm from the cauda epididymis and vas deferens were directly released into PBS. The collected cells were washed with PBS and then resuspended in sample buffer containing 3% SDS and boiled for 10 min.

Sperm Head and Tail Fractionation

Sperm from cauda epididymis and vas deferens were directly released and washed with 1× PBS. Collected sperm were resuspended in 2.5 ml of PBS containing 2 mM EGTA and 1 mM β-mercaptoethanol. After sonication for four-sec bursts, an equal volume of 1.8 M sucrose was added, and the suspension was layered over a discontinuous sucrose gradient containing equal volumes of 2.05 and 2.2 M sucrose solutions. The sample was centrifuged at 100,000 × g for 16 h at 4°C. The sperm heads and tails were collected from the pellet at the bottom and the middle of the 2.05 M sucrose layer, respectively [22]. The heads and tails were washed with 1× PBS and subjected to Western blot analysis; 1.8×106 heads and 9×105 tails were used for Western blotting.

In Vitro Fertilization

Cumulus cell-enclosed oocytes were collected from the oviductal ampulla of B6D2F1 mice superovulated by eCG and hCG. Thirty to 35 cumulus-enclosed oocytes were placed in a 0.1-ml drop of KSOM (lab made) covered with mineral oil. Sperm from cauda epididymis and vas deferens of 6-mo-old male mice were capacitated by incubation in M16 medium with 3% BSA at 37°C under 5% CO2 in air for 3 h. Subsequently, sperm were inseminated to medium containing eggs (5×105 sperm/ml). After 12 h of in vitro incubation, the female and male pronuclei in the eggs were stained with 4′-diamidino-2-phenylindole (1 μg/ml) for 15 min and then observed using a fluorescence microscope (Leica Microsystems).

Antibodies

The following antibodies were used in this study. The antibodies against several ADAM proteins were prepared as previously described [18, 19, 23]: rabbit-anti-ADAM4 (1/1000, lab made); rabbit-anti-ADAM5 (1/1000, lab made); rabbit-anti-ADAM6 (1/100, lab made); rabbit-anti-ADAM7 (1/1000, lab made); rabbit-anti-ADAM8 (1/1000, lab made); rabbit-anti-ADAM9 (1/1000, lab made); rabbit-anti-ADAM11 (1/1000, lab made); rabbit-anti-ADAM12 (1/1000, Millipore, MAB19292), rabbit-anti-ITM2B (1/3000, Abcam, ab14307), mouse-anti-ADAM3 (1/1000, Millipore, MAB19291), mouse-anti-HSPA5 (1/1000, Santa Cruz, H-129), rabbit-anti-CANX (1/1000, Stressgen, SPA-865), chicken-anti-ITM2B (1/5000, Abcam, ab14307), mouse-anti-α-tubulin (1/1000, Sigma, T6199), and mouse-anti-ADAM3 (1/1000, Millipore, 4G10). An affinity-purified rabbit polyclonal antibody to the cytoplasmic tail domains of mouse ADAM7 (1/1000, lab made) was prepared as previously described [15]. The rabbit polyclonal antibody to Mm.87328 (1/500, lab made) was prepared as previously described [22]. Secondary antibodies used in Western blot analysis were horseradish peroxidase (HRP)-conjugated goat anti-rabbit and anti-mouse IgG (Jackson Immunoresearch).

In Vitro Capacitation of Sperm

Capacitated sperm were prepared by releasing sperm from the caudal epididymis and vas deferens directly into prewarmed M16 medium (Sigma) containing 25 mM NaHCO3 and 5% bovine serum albumin (BSA; Sigma) and incubating for 90 min at 37°C in a 5% CO2 incubator. Uncapacitated sperm were prepared by incubating in medium without NaHCO3 or BSA. The sperm concentration in the medium was 2×105 cells/ml. The effectiveness of capacitation was enhanced by supplementing M16 medium with 3 mM pentoxifylline (Sigma), a phosphodiesterase inhibitor, and 5 mM dibutyryl cyclic adenosine monophosphate (Sigma), an analog of cAMP [24]. Methyl-β-cyclodextrin (Sigma), a specific cholesterol-binding reagent, was used for cholesterol depletion of sperm membranes [25, 26]. Sperm capacitation was monitored by observing sperm motility under an inverted light microscope (Leica Microsystems) during incubation. Uncapacitated and capacitated sperm were used for subsequent Western blot analyses.

Western Blot Analysis

Proteins were denatured by boiling for 10 min in the presence of 3% SDS and 5% β-mercaptoethanol, separated by SDS-PAGE, and transferred onto polyvinylidene difluoride membranes (0.2 μm; Bio-Rad Laboratories). After blocking with 5% nonfat dry milk in TBS-T (50 mM Tris-HCl pH 7.5, 150 mM NaCl [TBS] containing 0.1% Tween-20), the membranes were incubated with primary antibodies for 1 h. After three 10-min washes with TBS-T, membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room
temperature. Following three washes with TBS-T, immunoreactive proteins were detected using a chemiluminescence kit (Pierce).

Statistics

Results are presented as means ± SEM. The statistical significance of differences between data means was determined using Student t-test.

RESULTS

Generation of Adam7−/− Mice

To investigate the biological role of ADAM7 in vivo, we obtained Adam7-null mice, derived from embryonic stem cells in which the first and second coding exons were replaced with the IRES/βgeo/PolyA cassette via homologous recombination (Fig. 1A). Homologous recombination in the targeted embryonic stem cell clones was confirmed by genomic Southern blotting (Fig. 1, A and B). Chimeric and heterozygous (Adam7+/−) mice were generated using standard procedures. Homozygous (Adam7−/−) males were obtained by crossing with heterozygous mice. Adam7−/− mice were born from heterozygous parents with the expected Mendelian pattern of inheritance. Adam7+/+, Adam7+/−, and Adam7−/− mice were verified by PCR-based genotyping, in which 254- and 406-bp fragments represented the WT and mutant alleles, respectively (Fig. 1, A and C). Successful gene ablation of Adam7 was confirmed by the absence of the ADAM7 protein (108 kDa) in the epididymis from homozygous mice (Fig. 1D). All analyses reported here were performed using age-matched littersmates generated through heterozygous crosses. The Adam7−/− mice appeared to develop normally and showed no identifiable behavioral abnormalities or obvious adverse phenotypes.

Subfertility in Adam7−/− Males

To evaluate the function of ADAM7 in male reproduction, we performed a mating test. Males of each genotype were paired with virgin C57BL6/J females, and the number of litters produced per plugged female was monitored. No differences in mating behavior or vaginal plug formation were observed between Adam7−/− and Adam7+/− littersmates. However, females mated with Adam7−/− males showed a modest but significant reduction in litter size. The fertility rate of Adam7−/− males was reduced by ~15% relative to WT males (Table 1). Adult Adam7−/− females exhibited normal fertility (data not shown).

To corroborate the subfertility of Adam7−/− males, we performed a fertilization test using superovulated normal females mated with WT or Adam7−/− males. Eggs were collected from ampullae in the oviducts of the mated females, cultured, and examined for the presence of embryos cleaved to the two-cell stage to determine if fertilization had occurred (Fig. 2A, arrows). We observed that 76.2% ± 7.59% of eggs harvested from females impregnated by WT males were fertilized. In contrast, a reduced percentage (48.8% ± 4.11%) of eggs harvested from females mated with Adam7−/− males were fertilized (Fig. 2B). Collectively, our data demonstrate that the ability of Adam7−/− males to fertilize eggs in vivo is significantly compromised.

Altered Epididymal Integrity in Adam7−/− Male Mice

To further investigate male reproduction in the absence of ADAM7, we performed macroscopic examinations of the testis and epididymis, the major male reproductive organs. These examinations revealed no significant differences between WT and Adam7−/− males in gross morphology (Fig. 3, A and B).

Testis-to-body-weight and epididymis-to-body-weight ratios were similar between Adam7−/− and WT mice (Fig. 3, C and D). Since the epididymis expresses ADAM7, we further investigated this organ in Adam7−/− males. Remarkably, analysis of H&E-stained sections revealed that epithelial cell heights were dramatically decreased in segment I/segment II (Fig. 4, A–D) and modestly decreased in segment III (Fig. 4, E and F) in Adam7−/− caput regions. The cell heights in segment I/segment II of WT and Adam7-null mice were 43.9 ± 0.73 μm/100 tubules and 33.45 ± 0.91 μm/100 tubules, respectively (P < 0.0001). Consequently, the circumferences of the epididymal lumina were increased in Adam7−/− mice (Fig. 4, B and D) compared with WT mice (Fig. 4, A and C). However, the cell number of Adam7-null caput epithelium was similar to that of WT (Fig. 4, A and C, WT: 85.4 ± 4.24/50 tubules; B and D, Adam7-null: 90.5 ± 4.1 cells/50 tubules; P > 0.5). Additionally, we infrequently found histological abnormalities, such as sperm granulomas (Fig. 4D) and mineralization-like features (Fig. 4F), in the caput epididymis of Adam7−/− mice.

The absence of ADAM7 caused no alterations in epithelial cell height or luminal diameter in the corpus or cauda epididymides (Fig. 4, H, J, and K). However, hyperplasia (Fig. 4J) and vacuolation (Fig. 4K) were found in the cauda epididymis. The frequency of these abnormalities was low in Adam7-null cauda epididymis (18.82% ± 2.59%/100 tubules) but higher than that in WT epididymis (6.34% ± 0.53%/100 tubules; P < 0.05). Collectively, our histological analyses of WT and Adam7-null epididymides reveal a requirement for ADAM7 in maintaining the cellular integrity of epididymal tubules.

Changes in Structure and Function of Sperm from Adam7−/− Mice

To determine whether the failure of fertilization is associated with sperm number and/or changes in sperm quality in Adam7−/− mice, we evaluated sperm collected from the cauda epididymis and vas deferens of Adam7−/− mice and WT littersmates. Sperm number was not different between Adam7-null and WT mice (Fig. 5A). The appearance of the epididymal sperm from Adam7−/− mice was normal compared with those from WT mice (Fig. 5, B and E). In addition, the observed motility of Adam7−/− epididymal sperm was not different from that of WT sperm (Supplemental Movies S1 and S2 [Supplemental Data are available online]) and Fig. 6A).

Further, we examined the number, motility, and morphology of sperm obtained from the uterus of WT females mated with Adam7−/− or WT males. The number and motility of uterine sperm from Adam7−/− mice were similar to those from WT mice (Fig. 5C, data not shown). Interestingly, in contrast with the epididymal and vas deferens sperm, uterine sperm from Adam7−/− mice showed morphologically abnormal tails (Fig. 5, D and E). An abnormality seen frequently was sperm flagella with repeated bending, resulting in an angulated, zigzag pattern (Fig. 5D). To elucidate the exact localization of ADAM7 in mature sperm, head and tail fractions were prepared from epididymal sperm and analyzed by Western blotting. The Mm.87328 protein [22] and α-tubulin were used as controls localized in the heads and tails, respectively. We found that the ADAM7 protein is distributed in both the head and tail fractions (Fig. 5F). The ADAM7 signal was stronger in the tail fractions than the head fractions. Our results indicate that the ADAM7 protein is abundantly localized at the sperm tail and that the absence of ADAM7 leads to sperm tail deformations.

To further investigate sperm phenotypes in Adam7−/− mice, we incubated sperm from the cauda epididymis and vas
deferens in medium for sperm capacitation. In contrast with sperm observed immediately after collection from the tissues (Fig. 5, A and B), Adam7−/− sperm incubated in vitro exhibited motility significantly reduced compared with WT sperm (Supplemental Movies S3 and S4 and Fig. 6A) and morphologically abnormal tails (Fig. 6B). The abnormal morphology of the incubated sperm was similar to that shown in uterine sperm (Fig. 5D), but the frequency of abnormality was lower than that of uterine sperm (incubated sperm: 21.0% ± 2.58%, n = 3; uterine sperm: 34.6% ± 3.14%, n = 4). Protein tyrosine phosphorylation represents an important event in the in vitro sperm capacitation. We examined the protein

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**FIG. 1.** Generation of Adam7-knockout mice. A) Strategy for the generation of a targeted Adam7 allele in embryonic stem cells. Exons and introns are represented by numbered boxes and horizontal lines, respectively. Apa (A) and EcoRV (E) restriction sites, the 5'-external and 3'-internal probes used for Southern blot analysis, and regions for PCR genotyping (square) are indicated. The targeting vector contains a puromycin resistance gene (Puro') and a β-galactosidase-neomycin resistance gene (βgeo). The herpes simplex virus thymidine kinase gene is indicated as a white box. B) Southern blot analysis of WT (+/+) and targeted (+/−) embryonic stem cells. C) PCR genotyping analysis using allele-specific primers shown in A. Amplification yielded a 254-bp product in the WT allele, and a 406-bp product is in the knockout (KO) allele. D) Genotype confirmation of KO mice by Western blot analysis. Western blot analyses of epididymal samples from WT (+/+) and KO (−/−) mice were performed using an antibody against ADAM7. An anti-α-tubulin antibody was used as a control for sample loading.
tyrosine phosphorylation pattern of Adam7−/− sperm during capacitation. Like WT sperm, Adam7−/− sperm showed an increase in protein tyrosine phosphorylation across a wide range of sperm proteins during incubation in the capacitation medium (Fig. 6C). However, overall phosphotyrosine levels were lower in Adam7−/− sperm compared with those of WT sperm in both uncapacitated and capacitated conditions. To test the effect of these altered characteristics of Adam7−/− sperm on fertilization, we carried out in vitro fertilization assay using cumulus cell-enclosed eggs. We observed that most of cumulus cells did not fully disperse in the presence of Adam7−/− sperm (Supplemental Movie S6). The fertilization rate by Adam7−/− sperm was reduced by ~60% relative to WT sperm (23.03% ± 7.68% by WT sperm and 9.02% ± 1.35% by Adam7-null sperm, n = 3, P < 0.05, Student t-test). Our results suggest that altered motility, tail morphology, and tyrosine phosphorylation of Adam7−/− sperm are associated with the reduced in vitro fertilization rate.

Expression of ADAM7-Interacting Proteins and Other ADAMs in Adam7−/− Mice

We previously found that HSPA5, CANX, and ITM2B are associated with ADAM7 in mouse sperm membranes [18]. An additional feature of ADAM7 is that its level in sperm is dependent on the expression of ADAM2 and ADAM3 [19]. To investigate whether ADAM7 deletion affects the protein levels of interacting proteins and other ADAMs in sperm, we performed Western blot analyses on uncapacitated and capacitated sperm. It should be noted that previous reports have shown that ADAM7 association with ITM2B is promoted during the capacitation process [18]. We found that levels of HSPA5 and the long form of ITM2B but not CANX were decreased in both uncapacitated and capacitated sperm of Adam7−/− male, compared with WT males (Fig. 7A).

Interestingly, the level of the small, minor form of ADAM2 (~30 kDa) was also reduced in capacitated Adam7−/− sperm (Fig. 7B). Thus, our analysis of sperm proteins demonstrates that ADAM7 is critical for the normal expression of ADAM7-interacting and -related proteins in sperm.

**DISCUSSION**

In both mice and humans, ADAM7 is expressed in the epididymis and transferred to mature sperm [13, 15–17]. In the present study, we generated knockout mice lacking ADAM7 and found that fertility is partially but significantly decreased in the mutant male mice. Phenotypic analyses revealed impaired epididymal integrity and abnormal sperm motility and morphology. We previously found that mouse ADAM7 is localized to the tubules of all epididymal regions [15]. Our histological analyses of Adam7-null mice showed that the corpus and cauda epididymides were relatively undisturbed. However, these analyses revealed a drastic defect in the organization of the caput epithelium, characterized by a decrease in the height of the epithelium of the caput tubules. As a result, the high columnar epithelial cell morphology disappeared in the mutant caput. In the context of the epididymal defect in Adam7-null mice, a number of investigations of mice with mutations in epididymal genes exhibit abnormalities in epididymal development, structure, and function. These genes include the androgen receptor (Ar) [27]; c-ros tyrosine kinase receptor (Ros1) [28]; estrogen receptor α (Esr1) [29, 30]; nuclear receptor subfamily 1, group H, members 2 and 3 (Nr1h2 and Nr1h3) [31]; leucine-rich repeat domain containing G protein-coupled receptor 4 (Lgr4) [32, 33]; and milk fat globule-EGF factor 8 protein (Mfge8) genes [34, 35]. In particular, mice lacking Nrl1H2 and Nrl1H3 (Nr1h2/3−/−) exhibit a decrease in the thickness of the caput epithelium that results in regression of the polarized columnar-type secretory epithelium [31]—a defect very similar to that of Adam7−/− mice. NR1H2 and NR1H3 (also known as liver X receptors [LXRs]) are members of the nuclear receptor superfamily that bind DNA and regulate a variety of genes. Thus, it is possible that Adam7 gene expression in the caput is regulated directly or indirectly by NR1H2 and NR1H3. We do not know how the ablation of Adam7 leads to disrupted caput
epithelium organization. The height of epithelial cells is proportional to their protein synthesis and secretory activities. The caput epididymis is known to be very active in terms of apocrine secretion. Apocrine release is characterized by protrusion of a part of the apical blebs, and these blebs contain epididymosomes with cytoplasmic contents [4, 36, 37]. ADAM7 might be involved in negatively regulating the apocrine secretory processes in the caput by an unknown mechanism. In this case, the absence of ADAM7 could cause excessive secretory activity, leading to disrupted epididymal organization.

The epididymis provides the luminal environment necessary for maintaining normal sperm structure. Defects in the epididymal epithelium cause changes in the composition of the luminal environment, leading to altered sperm structure and function [5, 28, 31, 32, 38]. The disruption of the caput epithelium organization in Adam7-null mice resulted in reduced sperm motility and a change in the phosphorytrosine contents of sperm proteins. One of the major cellular changes during capacitation is abundant tyrosine phosphorylation of sperm tail proteins [39, 40]. We do not know the molecular mechanism underlying the relationship between ADAM7 and tyrosine phosphorylation. Perhaps ADAM7 is involved in the regulation of sperm membrane proteins that activate the tyrosine phosphorylation pathway in sperm.

Adam7-null mice produced abnormal sperm with bent or angulated flagella. In accordance with the tail deformation of Adam7-null sperm, we found that ADAM7 is present in the sperm tail as well as in the sperm head. Interestingly, a similar sperm tail phenotype was observed in mutant mice lacking ROS1 [28] or NRL1H2/3 [31]. As was the case in Adam7-null mice, the sperm flagella abnormality in these mice was correlated with defects in the organization of the caput epithelium. It has been proposed that the altered caput epithelium is related to impaired secretory and/or reabsorptive functions of the caput that affect sperm volume regulation and tail morphology [28, 31]. It should be noted that the tail deformation of Adam7-null sperm was most severe in the uterus, suggesting that the observed structural changes in Adam7 mutant sperm take place mainly during transit through the male-female reproductive tract. Mammalian sperm experience a natural decrease in osmotic pressure from the epididymis to the uterus [41, 42], and sperm lacking the sperm water channel protein aquaporin3 (Aqp3) are defective in osmoadaptation in the uterus, resulting in tail deformation [43]. It is possible that sperm exposed to an altered luminal environment in Adam7-null epididymis become defective in cell volume regulation, and the resulting flagellar abnormality manifests under hypotonic stress in the uterus. Mutant sperm with the abnormal tail structure could be impaired with respect to fertilization processes, such as sperm transport from the uterus to the oviduct [43].

To acquire modifications necessary to reach, recognize, and fuse with the female gamete, sperm leaving the testis must pass through the epididymal duct. These modifications involve sperm lipid remodeling during interaction of sperm with secretory proteins from the epithelial cells [3]. Previous studies have shown that ADAM7 is transferred from the epididymis to

![Image](https://example.com/image1.png)

**FIG. 3.** Phenotypic analyses of Adam7−/− mice. A) Macroscopic appearance of adult testes from an Adam7−/− mouse and a WT littermate (Adam7+/+). Bar = 2 mm. B) Macroscopic appearance of adult epididymides from Adam7−/− and Adam7−/− mice. Bar = 2 mm. C) Comparison of testis weight in Adam7−/− mice and WT littermates. Values are means ± SEM (P > 0.05, Student t-test). The average values of testis-weight-to-body-weight ratio of WT and Adam7−/− males were 0.29 ± 0.06 (n = 7) and 0.27 ± 0.03 (n = 13), respectively. D) Epididymis weight in Adam7−/− mice and WT littermates. Values are means ± SEM (P > 0.05, Student t-test). The average epididymis-weight-to-body-weight ratios for WT and Adam7−/− males were 0.12 ± 0.03 (n = 7) and 0.11 ± 0.01 (n = 13), respectively.
sperm and forms complexes with CANX, HSPA5, and ITM2B in sperm membranes [15, 17, 18]. In particular, ADAM7 and ITM2B are located in detergent-resistant regions that are highly correlated with membrane lipid rafts. Changes in ADAM7 conformation during the capacitation process markedly promote the association of ADAM7 with ITM2B [18]. In the present study, our analyses showed that levels of HSPA5, a member of the heat shock protein 70 (Hsp70) chaperone family

FIG. 4. Histological analyses of epididymides from adult Adam7−/− mice. Sections prepared from segment I (A, B), segment II (C, D), segment III (E, F), corpus (G, H), and cauda (I–K) epididymides of Adam7+/+ (A, C, E, G, and I) and Adam7−/− (B, D, F, H, J, and K) mice were stained with H&E. Decreased thickness of the epithelium, large dilation of the tubule (black asterisk), sperm granuloma (white asterisk), and mineralization-like deposits (arrow) were distinguishing features of the caput epididymis of Adam7−/− mice compared with WT mice. In the distal epididymis of Adam7−/− mice, hyperplasia (black arrowheads) and intraepithelial vacuoles (white arrowhead) were observed. The distal epididymis of Adam7−/− tissue exhibited a greatly increased frequency of intraepithelial vacuoles and hyperplasia. Bars = 100 µm (A–I) and 200 µm (J, K).
FIG. 5. Analyses of sperm from cauda epididymis, vas deferens, and uterus. A) Number of sperm in \textit{Adam7\textsuperscript{+/+}} and \textit{Adam7\textsuperscript{-/-}} mice. Sperm were collected from the cauda epididymis and vas deferens. The average number of mature sperm in WT and \textit{Adam7\textsuperscript{-/-}} males was $3.16 \pm 0.20 \times 10^7$ (n = 7) and $3.15 \pm 0.24 \times 10^7$ (n = 13), respectively (\(P > 0.05\), Student t-test). B) Microscopic appearance of cauda epididymal sperm from \textit{Adam7\textsuperscript{+/+}} and \textit{Adam7\textsuperscript{-/-}} mice. Bars = 10 \(\mu\)m. C) Number of uterine sperm from WT female mice mated with \textit{Adam7\textsuperscript{+/+}} and \textit{Adam7\textsuperscript{-/-}} male mice. Sperm were collected from the uterus after plug formation. The average number of uterine sperm from females mated with WT and \textit{Adam7\textsuperscript{-/-}} males was $2.90 \pm 0.18 \times 10^5$ (n = 4) and $1.95 \pm 0.37 \times 10^5$ (n = 6), respectively (\(P > 0.05\), Student t-test). D) Microscopic appearance of uterine sperm from \textit{Adam7\textsuperscript{+/+}} and \textit{Adam7\textsuperscript{-/-}} mice. The flagella of \textit{Adam7\textsuperscript{-/-}} uterine sperm showed angulation at various degrees or repeated bending (arrows). Bars = 10 \(\mu\)m. E) Comparison of sperm with abnormal tails in \textit{Adam7\textsuperscript{+/+}} and \textit{Adam7\textsuperscript{-/-}} mice. The frequency of abnormal tails, characterized by coiling, was dramatically increased in uterine sperm from \textit{Adam7\textsuperscript{-/-}} mice. The average percentages of sperm with abnormal flagella in cauda epididymis from WT and \textit{Adam7\textsuperscript{-/-}} males were $0.89\% \pm 1.08\%$ (n = 4) and $1.20\% \pm 1.43\%$ (n = 6), respectively. The average percentages of uterine sperm with abnormal flagella from WT and \textit{Adam7\textsuperscript{-/-}} males were $2.77\% \pm 0.76\%$ (n = 4) and $34.6\% \pm 3.14\%$ (n = 6), respectively (*\(P < 0.001\), Student t-test). F) Localization of ADAM7 on sperm head and tail. Sperm heads (H) and tails (T) were prepared and subjected to Western blot analysis (1.8 \(\times\) 10\(^6\) heads in H lane and 9 \(\times\) 10\(^5\) tails in T lane). Mm.87328 [22] and α-tubulin were included as control proteins.
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[44, 45], and the long form of ITM2B, a type II integral membrane protein of the BRI family [46], are decreased in sperm from Adam7−/− mice. The ADAM7 chaperone complex present in sperm membrane rafts might reflect the importance of the complex as a chaperone-stabilized functional unit [18, 47]. We also found that levels of the small form (∼30 kDa) of ADAM2 were decreased in sperm from Adam7−/− knockout mice. Mouse ADAM2 is present as a large precursor (100 kDa) in the testis and is processed to a 45-kDa protein in mature sperm [48]. Following the acrosome reaction, a subset of ADAM2 protein is further processed to a 27-kDa form [49]. The impaired ADAM7-ITM2B chaperone complex in sperm from Adam7-null mice might contribute to the disruption of sperm membrane remodeling during the capacitation process, as reflected in the reduced level of the ADAM2 processed form in capacitated sperm. Altered sperm membrane organization could be related to defects in sperm fertility.

In conclusion, we generated mice lacking ADAM7 and found that the protein is required for normal male fertility. Our results suggest that ADAM7 is critical for maintaining the integrity of epididymal structure, particularly the caput epithelium, which provides the appropriate luminal environ-
ment for normal sperm structure and functions. ADAM7 is also important for the assembly of a chaperone complex potentially involved in sperm functions during capacitation. ADAM7 is present in human epididymis and sperm, and a sperm tail abnormality is commonly found in infertile patients [50]. The information obtained from the present study in mice should provide valuable insights into the causes of infertility and subfertility in relevant clinical patients.

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