



HAL
open science

Germinal angiotensin I-converting enzyme is totally shed from the rodent sperm membrane during epididymal maturation

Sonia Metayer, Françoise Dacheux, J.L. Dacheux, Jean-Luc Gatti

► **To cite this version:**

Sonia Metayer, Françoise Dacheux, J.L. Dacheux, Jean-Luc Gatti. Germinal angiotensin I-converting enzyme is totally shed from the rodent sperm membrane during epididymal maturation. *Biology of Reproduction*, 2002, 67 (6), pp.1763-1767. 10.1095/biolreprod.102.006684 . hal-02680741

HAL Id: hal-02680741

<https://hal.inrae.fr/hal-02680741>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Germinal Angiotensin I-Converting Enzyme Is Totally Shed from the Rodent Sperm Membrane During Epididymal Maturation¹

Sonia Métayer, Françoise Dacheux, Jean-Louis Dacheux, and Jean-Luc Gatti²

Equipe "Spermatozoïdes," UMR 6073 INRA-CNRS, Institut National de la Recherche Agronomique, Centre de Nouzilly, 37380 Monnaie, France

ABSTRACT

Acquisition of sperm fertilizing ability is due, in part, to the reorganization of plasma membrane proteins that occurs during epididymal sperm transit. Using polyclonal antibodies against angiotensin I-converting enzyme (ACE), we showed that this enzyme is immunolocalized mainly on the middle piece of rat and mouse testicular sperm and with less intensity along the initial part of the principal piece of the flagellum. In both species, only some sperm from the caput epididymis were still reactive, whereas no labeling was observed on cauda epididymal sperm. The 105- to 110-kDa germinal ACE was absent from the rat testicular fluid but appeared in the fluid of the anterior epididymis. Thereafter, its molecular weight shifted to 94 kDa in the corpus epididymal fluid and remained at this weight in the caudal region. The 105- to 110-kDa immunoreactive protein was present in testicular rat sperm extract but was completely absent from epididymal sperm extracts. Western blot analysis of testicular and epididymal tissue extracts from the rat and mouse also confirmed that the germinal enzyme was absent from the epididymal sperm cell. Our results demonstrated that the rodent germinal ACE is released from the testicular sperm membrane when sperm enter the epididymis, a process similar to that observed in domestic mammals. This result is discussed in view of the suggested role for this enzyme in sperm fertility.

epididymis, gamete biology, male reproductive tract, sperm maturation, testis

INTRODUCTION

Sperm fertilizing ability results from their modifications in response to changes in the surrounding epididymal fluid [1–3]. Among these modifications, the sperm membrane surface is extensively remodeled by the integration, modification, or removal of proteins from the sperm membrane during epididymal transit [1, 2, 4, 5]. We have recently shown that the germinal form of the angiotensin I-converting enzyme (gACE) is one of these epididymis-processed proteins. This enzyme is liberated from the sperm membrane as it passes through the caput epididymis in various domestic mammals (ram, boar, stallion, and bull) [6]. This 105-kDa protein is retrieved in the fluid of the caput region, shows a subsequent shift to 94 kDa during epididymal transit, and remains at this molecular weight in the caudal fluid and in semen. The polyclonal antibody raised against the

caudal fluid ACE mainly immunolabeled the middle piece of the testicular and proximal caput of ram sperm. Only the acrosome was labeled on some sperm of the subsequent regions, indicating that part of the ACE remained inserted in the membrane in this species.

Several groups have obtained ACE-deficient transgenic mice in which both somatic ACE (sACE) and gACE were absent [7–9]. These animals had lowered blood pressure and renal defects, and the males showed greatly reduced fertility but normally motile sperm. More recently, it has been shown that the lack of germinal ACE is specifically responsible for this reduced male fertility [10, 11]. The precise stage at which the absence of ACE interferes with fertility has not been determined, but these sperm are unable to pass through the female genital tract or bind to the oocyte [7, 9]. A role has been suggested for ACE and angiotensin II in sperm capacitation and acrosome reaction processes [12–14], and the angiotensin AT1 receptor has been immunolocalized on the tail of rat and human sperm [15]. However, other results in human and domestic animals have indicated that this enzyme is not directly involved in the binding and fusion of the sperm with the oocyte [16–18]. We therefore extended our previous study on domestic mammals to laboratory models (mouse and rat) to determine whether germinal ACE is important for fertility within the male or female genital tract.

MATERIALS AND METHODS

Biological Materials

Epididymides were obtained from adult rats (Lewis; age, >6 mo) and mice (SV109; age, >3 mo). The organ was divided into five zones for rat fluids and sperm collection (Fig. 1) and into three zones (caput, corpus, and cauda) for rat and mouse tissue extraction. Rat testicular sperm were obtained after 24-h accumulation induced by ligation of the efferent duct [19]. The rat cauda epididymal fluid and spermatozoa were obtained from regions 4 and 5 by retroperfusion with PBS. The epididymal fluids and sperm from regions 1–3 were collected by gently extruding the epididymal content on a parafilm sheet. These fluids were mixed with an equal volume of PBS, and spermatozoa were separated by centrifugation (10 min, 15 000 × g). The supernatants were centrifuged again (10 min, 15 000 × g) and kept at –20°C until use. Sperm pellets were resuspended in PBS, washed by two cycles of centrifugation (10 min, 500 × g), and then extracted by mixing the last pellet with an equivalent volume of reducing sample buffer. After 5 min, the mixture was centrifuged (10 min, 15 000 × g), and the supernatant was then removed and heated for 5 min at 90°C and used directly or stored at –20°C.

For tissue extracts, the testes, kidneys, and various epididymal regions were carefully dissected from fat pad and gross blood vessels and rinsed in Dulbecco modified Eagle medium before grinding at 4°C in the same medium with 1% (w/v) SDS, a cocktail of protease inhibitors (Sigma, St. Quentin Fallavier, France), and 1 mM EDTA. The extract was then centrifuged (5 min, 2000 × g), and the supernatant was carefully removed and centrifuged twice at 15 000 × g (5 min). The final supernatant was diluted (1:1 v/v) with sample buffer, boiled, and either used immediately or stored at –80°C. The quantity of protein for each extract was equilibrated visu-

¹Supported by a grant from "Biotechnocentre-Région Centre." S.M. was the holder of a thesis grant from "Région Centre."

²Correspondence. FAX: 33 247 427 743; e-mail: gatti@tours.inra.fr

Received: 24 April 2002.
First decision: 14 May 2002.
Accepted: 25 May 2002.

© 2002 by the Society for the Study of Reproduction, Inc.
ISSN: 0006-3363. <http://www.biolreprod.org>

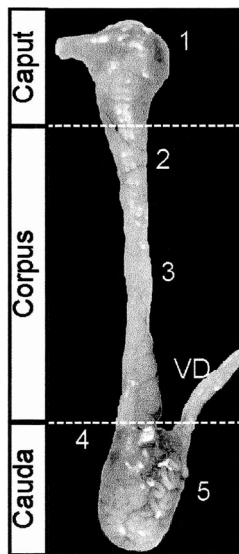


FIG. 1. Rat epididymis. Fluids and spermatozoa were collected at different sites of the epididymis: caput (zone 1), corpus (zones 2 and 3), and cauda (zones 4 and 5).

ally on a Coomassie blue-stained gel to be approximately 100 μg of protein per lane.

Gel Electrophoresis and Protein Blot Analysis

The sample preparation and electrophoretic methods used were as previously described [20]. Semidry transfer of proteins to nitrocellulose was performed over 2 h at 0.8 mA/cm². The blots were blocked with Tris-buffer saline-Tween containing 5% goat serum or nonfat dried milk. Two different rabbit polyclonal antibodies made in the laboratory against the gACE purified from the ram cauda epididymides were used in the present study either separately or together (final dilution, 1:5000) [6, 18]. These sera gave the same results on Western blot analysis. The polyclonal antibodies directed against intracellular C-terminal sequence were from the 28 series (28C8 and 28B8 [21]) and were a gift from Dr Alhenc-Gelas (U367, INSERM, Paris, France). They recognized only the complete membrane protein and the short sequence of approximately 10–15 kDa, which remains inserted in the membrane after ACE release from the cell [21]. They were used at a 1:2000 dilution. The secondary antibody was a goat anti-rabbit antibody conjugated with peroxidase (dilution, 1:5000; Sigma), was revealed with a chemoluminescent method (ECL+; Amersham Pharmacia Biotech, Uppsala, Sweden), and was visualized on film or by an imaging device. The second antibody used alone did not react on Western blots of the different samples.

Immunolocalization

Immunolocalization was performed as previously described [6, 22] on PBS-washed spermatozoa obtained either by collection after ligation of

testes or epididymal perfusion (rats) or from cut pieces of testis and epididymis (rats and mice). The cells were fixed and washed before the incubation with anti-ACE polyclonal antibodies (dilution, 1:500) and revealed with a goat anti-rabbit antibody labeled with fluorescein isothiocyanate (dilution, 1:100; Sigma). The fluorescence was photographed using a 400 ASA black-and-white film (Kodak, Rochester, NY). The second antibody used alone did not react significantly.

ACE Activity Measurement

The ACE activity was measured using rat caudal fluid as described previously [18, 23] with the furanacryloyl-L-phenylalanyl-glycylglycine substrate (FAPGG; Sigma). The ACE-inhibitor captopril was diluted from a 10⁻³ M stock solution in ethanol:dimethyl sulfoxide (1:1).

Chemicals

Molecular weight standards for electrophoresis were from Amersham Pharmacia Biotech. Reagents were of the best available grade from Sigma and ProLabo (Fontenay-sous-Bois, France).

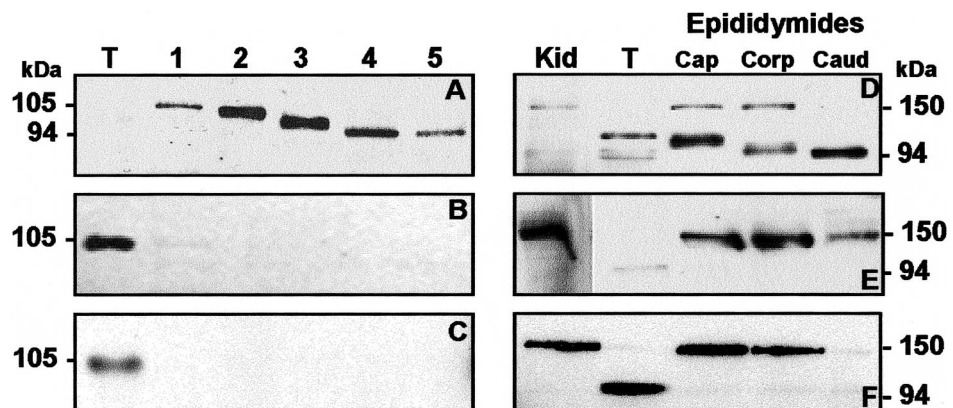
RESULTS

gACE and sACE in Rat and Mouse Epididymis

Proteins from the rat rete testicular fluid and from the fluid of the five different epididymal zones (Fig. 2) were separated by SDS-PAGE and transferred to nitrocellulose. The antibodies obtained against ram gACE (Fig. 2A) only reacted with a protein in the fluid from the epididymis. This protein of approximately 105–110 kDa in the proximal caput fluid (zone 1) showed a shift toward 94 kDa in the distal caput and corpus regions (zones 2 and 3). The reactive protein remained at this size in the cauda epididymides, although its concentration appeared to decrease (zones 4 and 5) and a carboxypeptidase activity of 6.4×10^{-2} μmoles FAPGG/(min and mg of protein) was found in this caudal fluid. This activity was totally inhibited by 10⁻⁴ M captopril, a specific and irreversible inhibitor of ACE. This activity copurified with the 94-kDa immunoreactive protein on gel filtration and ion-exchange chromatography (not shown). The immunoreactive protein was never observed in the rat rete testicular fluid collected from three different animals, and no carboxypeptidase activity was measured in these testicular fluids.

The same anti-ram gACE antibodies reacted specifically with a 105- to 110-kDa compound with the rat testicular sperm extracts and, with much less intensity, on sperm extract from zone 1 (Fig. 2B). No reaction was observed in the sperm extract from the subsequent epididymal regions (Fig. 2B). The same sperm extracts were also probed with polyclonal antibodies (28C8 and 28B8) directed against the C-terminal sequence of the sACE, which recognize the

FIG. 2. ACE reactivity in epididymis. Rat epididymal fluids (A) and sperm plasma membrane extracts (B and C) from the testis (T) and different epididymal zones (1–5) as well as rat (D and E) and mouse (F) proteins extracted from kidney (Kid) and testis, caput, corpus, and cauda epididymal regions were separated on 6%–16% SDS-PAGE and subjected to Western blot analysis. The blots were incubated either with ram anti-gACE polyclonal antibodies (A, B, and D) or with anti-C-terminal ACE antibodies (C, E, and F).



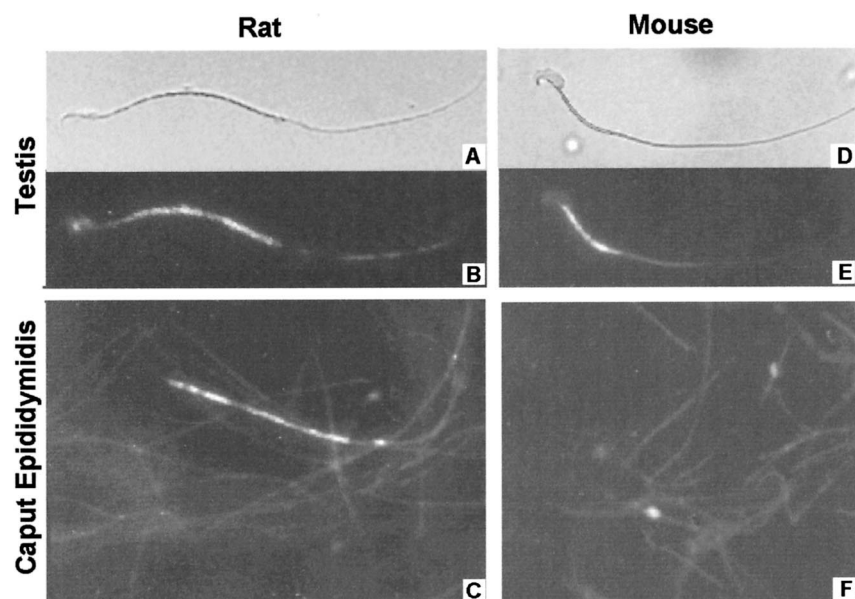


FIG. 3. ACE immunolocalization on rat and mouse sperm. Rat and mouse testicular and caput epididymal sperm were incubated with 28B8 anti-C-terminal ACE antibody and revealed with a secondary fluorescent antibody. **A** and **D**) Same as **B** and **E** in bright field. **B**, **C**, **E**, and **F**) Fluorescence. Scale: 12 mm = 10 μ m.

complete (i.e., uncleaved) ACE. Both antibodies gave the same results (Fig. 2C); that is, they recognized a band at 105–110 kDa only in the testicular sperm extract representing the complete gACE (Fig. 2C). No reaction occurred with the sperm extracts from the other epididymal regions.

We also probed tissue extracts obtained from the kidney, testis, and the three main regions of the epididymis either with the polyclonal antibodies against ram gACE or the C-terminal region (Fig. 2, D and E, respectively). The anti-ram gACE antibodies reacted with the 150- to 160-kDa sACE present in the kidney extract (Fig. 2D). The 105- to 110-kDa band, representing gACE, was clearly reactive in the testicular extract, and in some cases, a faint band could also be detected at 150–160 kDa. A lower molecular weight band at approximately 90 kDa was also visible and could represent the intracellular nonglycosylated form of the gACE. Both the somatic 150- to 160-kDa form and the germinal form, showing the shift from 105–110 to 94 kDa, were reactive in the caput and corpus epididymal extracts, whereas mainly the germinal form was visible in the caudal extract. The same tissue extracts were probed with anti-C-terminal antibodies to determine the membrane-bound form of ACE (Fig. 2E). Only the somatic 150- to 160-kDa form reacted in the kidney and the three epididymal regions extracts, whereas it was mainly the 105- to 110-kDa form in the testicular extract. This confirmed that the gACE had lost its C-terminal anchor and was transported as a soluble enzyme in the epididymis.

Using the C-terminal antibody 28B8, which was the most reactive antibody in the mouse, we probed the tissue extracts obtained from mouse kidney, testis, and epididymis (Fig. 2F). The antibody recognized the 150- to 160-kDa sACE form in the kidney, caput, and corpus epididymis and the 105- to 110-kDa germinal form in the testis. No membrane-bound germinal form could be observed in the epididymal extracts, suggesting that the enzyme disappeared from the sperm membrane as previously observed in the rat. Unfortunately, our anti-ram gACE did not cross-react with the mouse sACE or gACE.

Immunolocalization of gACE on Rat and Mouse Sperm

We used the anti-C-terminal antibody 28B8 to immunolocalize gACE on rat and mouse sperm membranes (Fig.

3). In both species, the antibody clearly labeled the mid-piece of the testicular sperm and, with less intensity, the beginning of the principal piece of the flagellum (Fig. 3, B and E). In the caput epididymis, only few sperm (estimated at <5%) showed the same labeling (Fig. 3C). When the entire caput was used to retrieve sperm in mouse, almost no reactive sperm could be found (Fig. 3F). Only when sperm were taken from the proximal caput were some of them reactive (not shown). No labeling was observed with sperm from the cauda epididymis in either species.

DISCUSSION

In the male, two isoforms of ACE are derived from the same gene [24]. One is a somatic isoform that is constituted by two homologous N- and C-catalytic domains and has a molecular weight of 150–180 kDa. This isoform is involved in the regulation of blood pressure and is widely distributed in many tissues in the body [24, 25]. The other is a germinal isoform (also known as testicular) that is found exclusively on the haploid germ cell in the testis [26]. This germinal form of approximately 110 kDa is restricted to the C-terminus domain of the somatic enzyme due to the presence of a specific testis-promoter within the 12th intron of the gene. This germinal isoform is also characterized by a species-specific N-terminal [24]. The common C-terminal domain of these two isoforms contains a hydrophobic amino acid sequence allowing cellular membrane insertion. The somatic cell-bound ACE can become a free, blood-circulating enzyme after proteolytic cleavage of this anchor (for review, see [27]).

Using biochemistry and immunochemistry, we showed in the present study that gACE was present only on the membrane of mouse and rat testicular sperm and that the protein was completely released as the sperm passed through the proximal caput of the epididymis. The gACE was immunolocalized on the middle piece and, to a lesser extent, on the principal piece of the flagellum, a location identical to that described in the ram. Similar immunolocalization was obtained using the anti-ram gACE polyclonal antibodies on rat testicular sperm, although these antibodies also labeled the acrosome (not shown).

This removal of gACE from the sperm membrane is very similar to that observed in domestic mammals, where

it also occurs in the proximal part of the caput epididymis, suggesting the need for a specific environment for this process. In the reproductive tract, several sperm membrane proteins have been shown to be processed proteolytically in the caput epididymis during transit [28–30], and several proteases have been described in the fluid [31, 32]. However, to our knowledge, the protease involved in the precise mechanism of ACE liberation has not yet been identified.

Once liberated, the gACE remained as far as the rat cauda epididymal fluid, but a change in its molecular weight from 105 to 94 kDa occurred in the corpus, a change that has also been reported in domestic mammals [6]. This could be due to variations in postsecretory modifications, because as revealed by the presence of several spots with pIs between 6.0 and 8.0 on two-dimensional separation (not shown), the rat caudal fluid gACE is certainly glycosylated. We attempted to purify the germinal ACE of the rat cauda epididymal fluid, but the quantity of protein obtained was very low (not shown) and was partly masked by the 97-kDa β -D-galactosidase [33], which copurified with the ACE.

It has been suggested that male mice “knocked out” for ACE have very low fertility because their sperm are unable to pass properly through the female genital tract (although they are perfectly motile) and they cannot bind to the oocyte during *in vitro* fertilization [7, 9]. Our findings suggest that the sperm physiological changes may not relate to a direct role of ACE in capacitation, acrosomal reaction, or sperm-oocyte binding, because ejaculated mouse sperm may have no remaining ACE. The hypothesis that sperm ACE is not involved in postejaculatory fertilization stages is also supported by the fact that oocyte fertilization in other mammals is not altered by the presence of antibodies against this enzyme [16] or by the presence of the ACE-inhibitor captopril [17, 18].

It is interesting to note that the male mice knocked out for fertilin alpha and cyritestin, which are also testicular sperm proteins processed during epididymal maturation, and for calmeglin, which is a chaperone involved in the processing of membrane protein (for review, see [34]), have almost the same phenotype as ACE knock-out mice: a decrease in the number of sperm able to pass through the female genital tract and/or sperm binding to the oocyte, without alteration in sperm production or motility. Moreover, recent results have indicated that knock-out of fertilin and cyritestin induced a decrease in or disappearance of several other membrane components [35]. Considered together, these findings suggest that changes in one of the components of the sperm membrane produce a nonspecific phenotype and may be due to an impairment of maturation of the membrane domains during spermiogenesis or epididymal transit. The absence of ACE from the testicular sperm therefore may, in fact, produce slight changes in the sperm maturation process without affecting sperm motility. However, we cannot completely exclude the possibility that the ACE knockout phenotype may also be related to the enzymatic function of this protein in the epididymis [36]. However, the fact that sACE cannot restore fertility of the sperm of gACE^{-/-} mice does not support this last hypothesis [11]. Moreover, angiotensin II does not seem to be involved in the gACE^{-/-} sperm phenotype, because male mice without angiotensinogen and, thus, angiotensin apparently remain fertile [37]. It is also of note that in this species, gACE is released in an epididymal area, where the tissue is already very rich in sACE protein (see *Results*).

In conclusion, we have demonstrated in the rat and

mouse that the germ cell membrane-bound ACE is completely released under an active form and that this release occurs in a very precise epididymal area, a process similar to that observed in other mammalian species. The exact role played by this enzyme in the sperm reproductive function has yet to be elucidated, but the present findings suggest that its absence may impair the building or remodeling of the sperm membrane domains.

ACKNOWLEDGMENTS

The authors thank Dr. Alhenc-Gelas (U367, INSERM) for the gift of anti-C-terminal ACE antibodies and helpful discussions. The technical help of Mrs. A. Collet and the staff of the rodent experimental unit of the Physiologie de la Reproduction et des comportements is also gratefully acknowledged.

REFERENCES

1. Yanagimachi R. Mammalian fertilization. In: Knobil E, Neill JD (eds.), *The Physiology of the Reproduction*, 2nd ed. New York: Raven Press; 1994: 189–317.
2. Turner TT. On the epididymis and its role in the development of the fertile ejaculate. *J Androl* 1995; 16:292–298.
3. Dacheux JL, Druart X, Fouchécourt S, Syntin P, Gatti JL, Okamura N, Dacheux F. Role of epididymal secretory proteins in sperm maturation with particular reference to the boar. *J Reprod Fertil Suppl* 1998; 53:99–107.
4. Voglmayr JK, Sawyer RF, Dacheux J-L. Glycoproteins: a variable factor in surface transformations of ram spermatozoa during epididymal transit. *Biol Reprod* 1985; 33:165–176.
5. Dacheux J-L, Dacheux F, Paquignon M. Changes in sperm membrane and luminal proteins fluid during epididymal transit in the boar. *Biol Reprod* 1989; 40:635–651.
6. Gatti JL, Druart X, Guérin Y, Dacheux F, Dacheux JL. A 105- to 94-kilodalton protein in the epididymal fluids of domestic mammals is angiotensin I-converting enzyme (ACE): evidence that sperm are the source of this ACE. *Biol Reprod* 1999; 60:937–945.
7. Kregel JH, John SWM, Langenbach LL, Hodgkin JB, Hagaman JR, Bachman ES, Jenette JC, O'Brien DA, Smithies O. Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature* 1995; 375:146–148.
8. Esther CR, Marino EM, Bernstein KE. The role of angiotensin-converting enzyme in blood pressure control, renal function, and male fertility. *Trends Endocrinol Metab* 1997; 8:181–186.
9. Hagaman JR, Moyer JS, Bachman ES, Sibony M, Magyar PL, Welch JE, Smithies O, Kregel JH, O'Brien DA. Angiotensin-converting enzyme and male fertility. *Proc Natl Acad Sci U S A* 1998; 95:2552–2557.
10. Ramaraj P, Kessler SP, Colmenares C, Sen GC. Selective restoration of male fertility in mice lacking angiotensin-converting enzymes by sperm-specific expression of the testicular isozyme. *J Clin Invest* 1998; 102:371–378.
11. Kessler SP, Rowe TM, Gomos JB, Kessler PM, Sen GC. Physiological nonequivalence of the two isoforms of angiotensin-converting enzyme. *J Biol Chem* 2000; 275:26259–26264.
12. Wong PYD, Uchendu CN. The role of angiotensin-converting enzyme in the rat epididymis. *J Endocrinol* 1990; 125:457–465.
13. Vinson GP, Saridogan E, Puddefoot JR, Djanhanbakhch O. Tissue renin-angiotensin systems and reproduction. *Hum Reprod* 1997; 12: 651–662.
14. Köhn F-M, Müller C, Drescher D, Neukamm C, El Mulla KF, Hagele W, Hinsch E, Habenicht UF, Schill W-B. Effect of angiotensin converting enzyme (ACE) and angiotensins on human sperm functions. *Andrologia* 1998; 30:207–215.
15. Vinson GP, Puddefoot JR, Ho MM, Barker S, Mehta J, Saridogan E, Djanhanbakhch O. Type 1 angiotensin receptors in rat and human sperm. *J Endocrinol* 1995; 144:369–378.
16. Dobrinsky I, Ignatz GG, Fagnan MS, Yudin AI, Ball BA. Isolation and characterization of a protein with homology to angiotensin converting enzyme from the periacrosomal plasma membrane of equine spermatozoa. *Mol Reprod Dev* 1997; 48:251–260.
17. Köhn F-M, Dammshäuser I, Neukamm C, Renneberg H, Siems W-E, Schill W-B, Aumüller G. Ultrastructural localization of angiotensin-converting enzyme in ejaculated human spermatozoa. *Hum Reprod* 1998; 13:604–610.

18. Métayer S, Dacheux F, Guérin Y, Dacheux J-L, Gatti J-L. Physiological and enzymatic properties of the ram epididymal soluble form of germinal angiotensin I-converting enzyme (ACE). *Biol Reprod* 2001; 65:1332–1339.
19. Tuck RR, Setchell BP, Waites GM, Young JA. The composition of fluid collected by micropuncture and catheterization from the seminiferous tubules and rete testis of rats. *Pflug Arch* 1970; 318:225–243.
20. Syntin P, Dacheux F, Druart X, Gatti JL, Okamura N, Dacheux JL. Characterization and identification of proteins secreted in the various regions of the adult boar epididymis. *Biol Reprod* 1996; 55:956–974.
21. Wei L, Alhenc-Gelas F, Soubrier F, Michaud A, Corvol P, Clauser E. Expression and characterization of recombinant human angiotensin I-converting enzyme. Evidence for a C-terminal transmembrane anchor and for a proteolytic processing of the secreted recombinant and plasma enzymes. *J Biol Chem* 1991; 266:5540–5546.
22. Okamura N, Dacheux F, Venien A, Onoe S, Huet JC, Dacheux JL. Localization of a maturation-dependent epididymal sperm surface antigen recognized by a monoclonal antibody raised against a 135-kilodalton protein in porcine epididymal fluid. *Biol Reprod* 1992; 47:1040–1052.
23. Holmquist B, Bünning P, Riordan JF. A continuous spectrophotometric assay for angiotensin converting enzyme. *Anal Biochem* 1979; 95:540–548.
24. Sibony M, Gasc J-M, Soubrier F, Alhenc-Gelas F, Corvol P. Gene expression and tissue localization of the two isoforms of angiotensin I-converting enzyme. *Hypertension* 1993; 21:827–835.
25. Peach MJ. Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol Rev* 1977; 57:313–370.
26. Sibony M, Segretain D, Gasc JM. Angiotensin-converting enzyme in murine testis: step-specific expression of the germinal isoform during spermiogenesis. *Biol Reprod* 1994; 50:1015–1026.
27. Hooper NM, Karran EH, Turner AJ. Membrane protein secretases. *Biochem J* 1997; 321:265–279.
28. Petruszac JAM, Nehme CL, Bartles JR. Endoproteolytic cleavage in the extracellular domain of the integral plasma membrane protein CE9 precedes its redistribution from the posterior to the anterior tail of the rat spermatozoon during epididymal maturation. *J Cell Biol* 1991; 114:917–927.
29. Jones R, Ma A, Hou S-T, Shalgi R, Hall L. Testicular biosynthesis and epididymal endoproteolytic processing of rat sperm surface. *J Cell Science* 1996; 109:2561–2570.
30. Hunnicutt GR, Koppel DE, Myles DG. Analysis of the process of localization of fertilin to the sperm posterior head plasma membrane domain during sperm maturation in the epididymis. *Dev Biol* 1997; 191:146–159.
31. Lum L, Blobel CP. Evidence for distinct serine protease activities with a potential role in processing the sperm protein fertilin. *Dev Biol* 1997; 191:131–145.
32. Métayer S, Dacheux F, Guérin Y, Dacheux J-L, Gatti J-L. Comparison, characterization, and identification of proteases and protease inhibitors in epididymal fluids of domestic mammals: matrix metalloproteinases are major fluid gelatinases. *Biol Reprod* 2002; 66:1219–1229.
33. Tulsiani DR, Skudlarek MD, Nagdas SK, Orgebin-Crist MC. Purification and characterization of rat epididymal-fluid α -D-mannosidase: similarities to sperm plasma-membrane α -D-mannosidase. *Biochem J* 1993; 290:427–436.
34. Evans JP. Fertilin beta and other ADAMs as integrin ligands: insights into cell adhesion and fertilization. *Bioessays* 2001; 23:628–639.
35. Nishimura H, Cho C, Branciforte DR, Myles DG, Primakoff P. Analysis of loss of adhesive function in sperm lacking cyritestin or fertilin beta. *Dev Biol* 2001; 233:204–213.
36. Chan HC, Wong PYD. Paracrine-autocrine regulation of anion secretion in the epididymis: role of angiotensin II. *Biol Signals* 1996; 5:309–316.
37. Tempfer CB, Moreno RM, Gregg AR. Genetic control of fertility and embryonic waste in the mouse: a role for angiotensinogen. *Biol Reprod* 2000; 62:457–462.