GENERAL INTRODUCTION

Fertilization is a unique process whereby two gametes from female (egg) and male (sperm) unite to produce offspring whose genetic makeup is different from both parents. The bisexual mode of reproduction *via* fertilization has emerged during evolution and has been maintained in most metazoans including mammals. Moreover, this process is totally essential to generate a wide variety of species.

Sperm is a highly specialized, differentiated cell to transfer the paternal genetic information to egg (1). The plasma membrane of mammalian sperm has different regions, which are probably related to different functions: an acrosomal region with exocytosis, equatorial and postacrosomal regions with gamete fusion, a mid-piece region with energy supply, and a tail region for locomotion (Fig. 1). The sperm acrosome is an intracellular membrane-limited organelle that surrounds the anterior portion of the nucleus (Fig. 1). This secretory vesicle consists of an inner acrosomal membrane that is closely associated with portion of the nucleus and an outer acrosomal membrane underlying the plasma membrane of the sperm head, and contains acrosomal proteins that make up a matrix. (Fig. 1). Ultrastructurally, this matrix is highly electron-dense and observed as a paracrystalline material. A variety of enzymes, including proteases, glycosidases, phosphatases, and phospholipases, are present in the acrosome, and some of them may be essential components for fertilization. Also, the acrosome is formed by aggregation of electron-dense granules, proacrosomal granules, derived from Golgi-apparatus during spermiogenesis (2). Synthesis of acrosomal proteins begins as early as the pachytene stage of meiosis, although the acrosome vesicle is not yet formed (1). The size and shape of the acrosome considerably vary from species to species (Fig. 1).

The steps of mammalian fertilization, including the acrosome reaction, are considered as follows: (Fig. 2 and see reviews 1, 3-7).

1) Sperm first associate with zona pellucida (ZP), extracellular glycoprotein surrounding ovulated egg. This association (termed attachment) between sperm and egg is relatively loose and nonspecific.

2) Then, sperm is able to form a relatively tight, species-specific association with egg ZP (primary binding). This binding is mediated by putative receptors present in

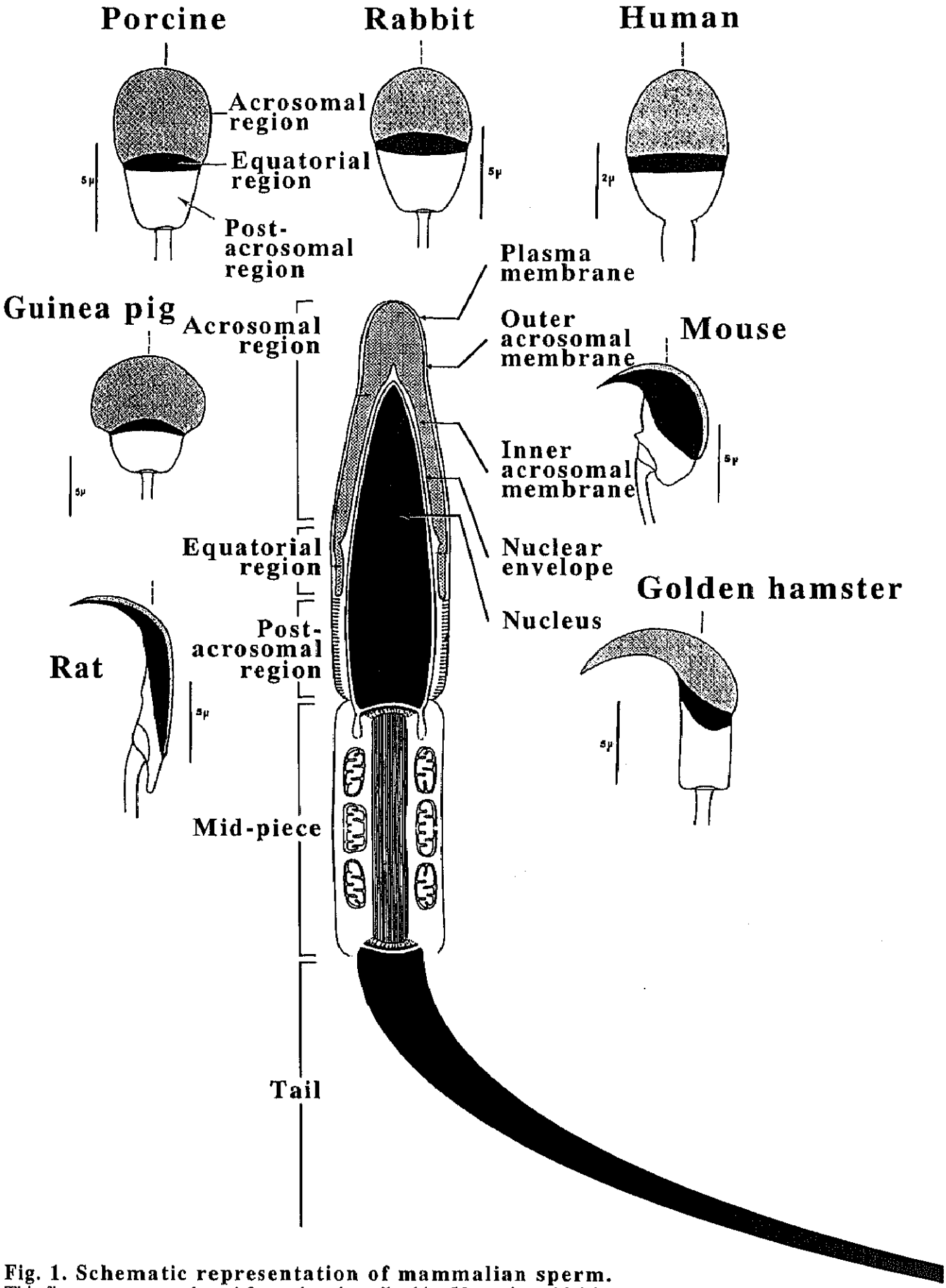

Fig. 1. Schematic representation of mammalian sperm.
This figure was reproduced from that described by Yanagimachi (1).

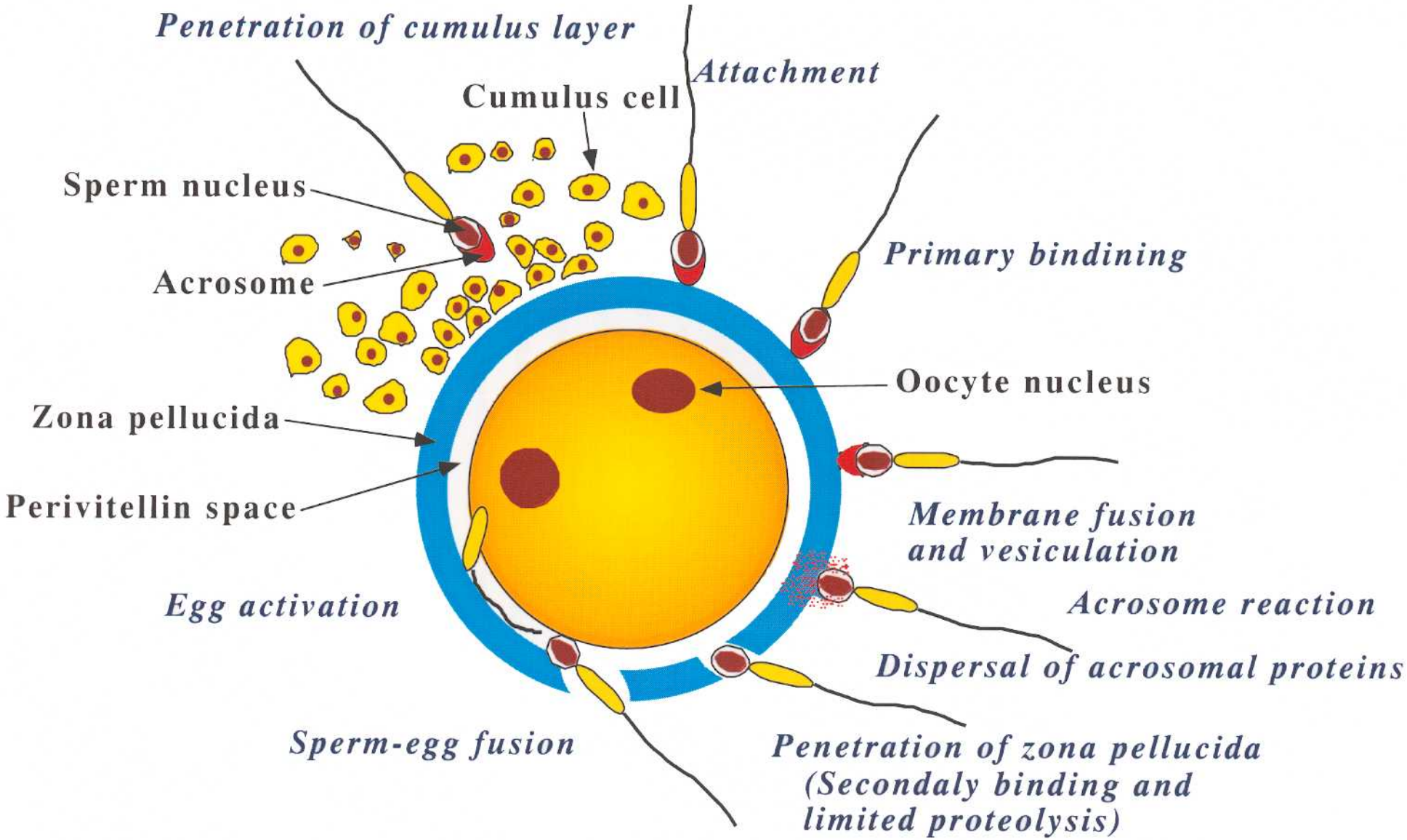

Fig. 2. Schematic diagram of the fertilization in mouse.

the ZP and complementary egg-binding proteins present in the sperm plasma membrane (8-14).

3) Sperm cause cellular exocytosis, acrosome reaction, stimulated by ZP3, one of three glyoproteins of the ZP (15-17). The acrosomal components are dispersed and inner acrosomal membrane is exposed.

4) Acrosome-reacted sperm is capable to penetrate the ZP, apparently by using various hydrolytic enzymes, including trypsin-like protease(s) dispersed from the acrosome. During sperm penetration through the ZP, sperm also bind to the ZP (secondary binding).

5) Sperm, which have reached the space (perivitelline space) between the ZP and egg plasma membrane, can then fuse with the egg. Fusion occurs between plasma membranes of the sperm and egg.

6) Sperm entered into the egg cytoplasm cause the egg activation for preparation of embryonic development.

Morphologically, the acrosome reaction occurs in several steps: (i) fusion of the outer acrosomal membrane with the overlaying plasma membrane, (ii) vesiculation and disappearance of the fused membranes, and (iii) dispersal of hydrolytic enzymes and other components present within the acrosomal matrix (Fig. 3 and ref. 1).

It is conceivable that sperm must be acrosome-reacted to effect penetration of the ZP. However, the exact position where the sperm cause the acrosome reaction has not been clarified and appears to differ among species (18-28).

Acrosin is one of acrosomal enzymes that have been most extensively characterized. This enzyme can be classified into a trypsin-like serine protease by its substrate specificity (29, 30) and inhibition profile toward various protease inhibitors (31). To date, acrosin have been identified in the sperm extracts from various mammalian species, including human, porcine, and mouse (32-41). Moreover, Sawada and coworkers (42) have successfully purified acrosin from ascidian sperm. The primary structures of mammalian acrosins have been deduced from the cDNA sequences (ref. 43, 44 for mouse, 45 for rat, 46, 47 for human, , 48, 49 for porcine, 50 for rabbit, 51 for bovine, 52 for guinea pig). Therefore, it can be considered that acrosin is one of the most essential molecules for fertilization because of its wide-spread distribution.

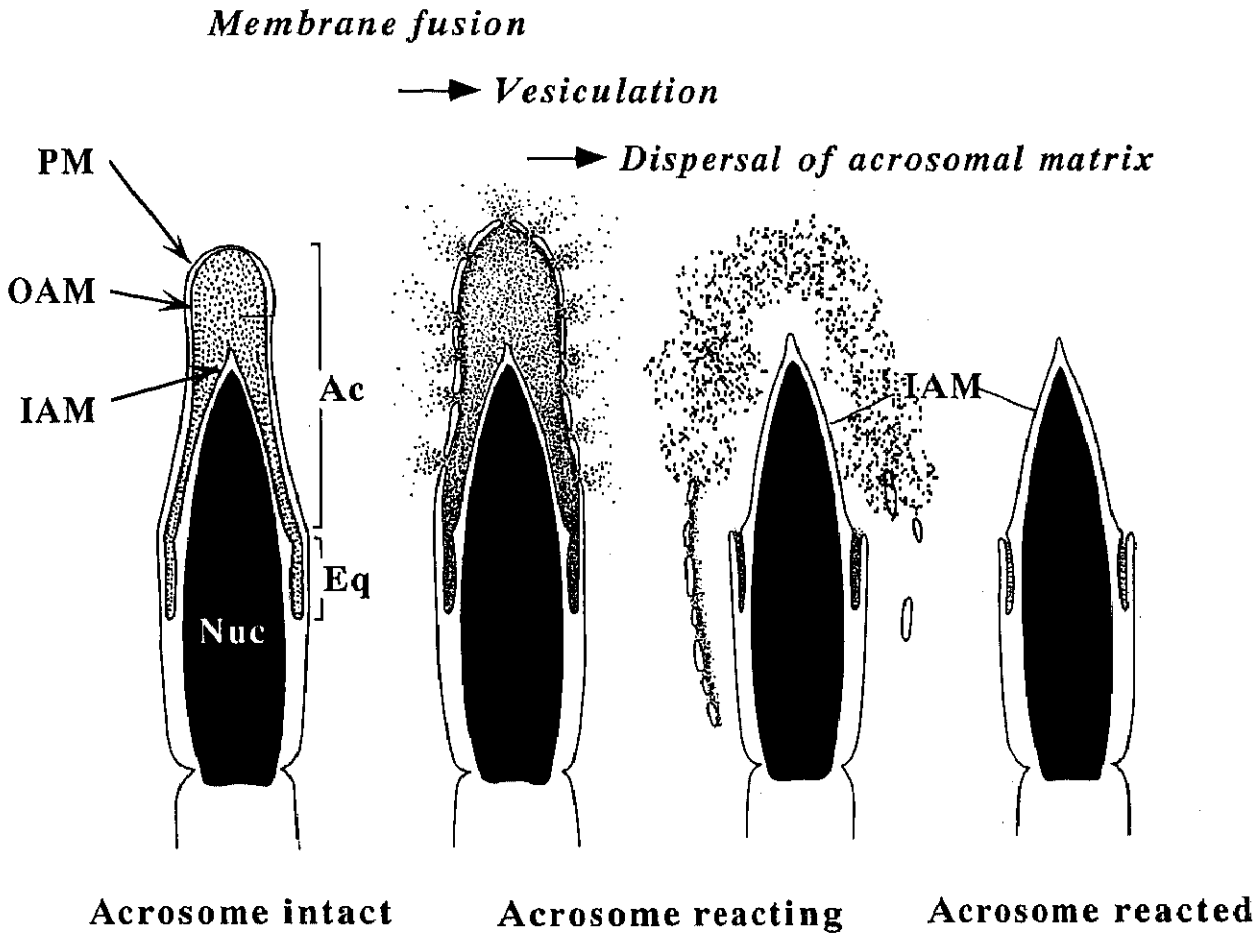

Fig. 3. Schematic diagram of acrosome reaction in mammalian sperm. This figure is reproduced from that described by Yanagimachi (1). *PM*, plasma membrane; *OAM*, outer acrosomal membrane; *IAM*, inner acrosomal membrane; *Ac*, acrosomal region; *Eq*, equatorial region.

Acrosin is specifically present in acrosomal matrix as an enzymatically inactive zymogen, proacrosin. The conversion of proacrosin to the active form, acrosin, is taken place during the time of acrosome reaction (34, 53-56). This process occurs spontaneously by autoactivation triggered by disruption of acrosome (57, 58), and has been extensively studied using porcine proacrosin (48, 59-62).

Several observations have led us to consider that acrosin has multiple functions in fertilization. It has been thought to act on (i) primary binding of acrosome-intact sperm to ZP (63), (ii) fusion event between outer acrosomal membrane and overlying plasma membrane (64-68), (iii) dispersal of acrosomal proteins during acrosome reaction (53, 69-71), (iv) limited proteolysis of the ZP (72-81) by its proteolytic activity. Moreover, acrosin may be involved in (v) secondary binding of acrosome-reacted sperm to the ZP (82-93), and also in (vi) sperm-egg fusion (94, 95) (Table I). The importance of acrosin in the sperm penetration of the ZP has been proposed by two lines of experiments. One is the prevention of fertilization and the sperm penetration through ZP (96-103) by using natural and synthetic inhibitors for trypsin and acrosin. Another is the capacity of acrosin for degradation of the ZP (72-81).

Recently, using homologous recombination, Baba and coworkers (104) have successfully produced male mice carrying a disruptive mutation in the acrosin gene, acrosin-deficient mice. The acrosin-deficient mouse sperm completely lacking the acrosin protease activity still penetrated ZP and normally fertilized the egg. A unique phenotype observed in the acrosin-deficient mouse sperm showed a delay in sperm penetration of the ZP solely at the early stages after insemination, as compared with homozygous and heterozygous mice sperm (Fig. 4). These data provide evidence that acrosin is not essential for sperm penetration of the ZP, leading to two additional questions:

(i) What is the actual function of acrosin in fertilization?

(ii) Does the sperm acrosome still contain trypsin-like protease(s), other than acrosin, that act on sperm penetration through ZP?

To answer these two questions, I have studied the roles of sperm serine proteases by using the acrosin-deficient mouse sperm. In CHAPTER I, I have analyzed the phenotypes of the acrosin-deficient mouse sperm to examine the actual role of acrosin

Table I. Summary of Physiological Functions of Acrosin in Fertilization.

Functions	Experimental Design	Species	References
Fertilization	Natural trypsin inhibitors, IVF assay	Rabbit	(96), 1969 (97), 1971
		Ram	(98), 1973
		Mouse	(99), 1979
	Synthetic trypsin inhibitors, IVF assay	Mouse	(100), 1982
	Thiol protease inhibitors, IVF assay	Hamster	(101), 1984
	Acrosin activity of infertile patients	Human	(102), 1982 (103), 1989
Acrosome reaction			
Membrane fusion	Synthetic trypsin inhibitors, ultrastructure	Hamster	(64), 1976 (65), 1979 (66), 1984
		Human	(68), 1993
	ā-Acrosin mAb, immunohistochemistry	Human	(67), 1990
Dispersal of acrosomal proteins	Synthetic trypsin inhibitors, ultrastructure	Guinea pig	(53), 1978 (70), 1982
	Synthetic trypsin inhibitors, morphology, ultrastructure	Mouse	(69), 1982
	Acidic pH, synthetic and natural inhibitors, morphology, ultrastructure	Guinea pig	(71), 1985
Penetration through ZP			
Limited proteolysis of ZP	Partialy purified acrosin, intact ZP	Rabbit	(72), 1969
		Hamster	(73), 1976
	Ram acrosin, various species ZP	Ram, Sheep, Porcine, Mouse, Gerbil	(74), 1982
	Purified acrosin, solubilized ZP	Porcine	(75) (76) (77) (78), 1985 (80), 1989
	Ram and porcine acrosin, mouse and sheep ZP	Ram, Porcine, Mouse, Sheep	(79), 1986
	Caprine acrosin, porcine ZP	Caprine, Porcine	(81), 1989
Secondary binding	Purified acrosin, fucoidan	Porcine	(82), 1987
	Sperm crude extract, labeled ZP and fucose	Porcine	(83), 1988
	Purified ZP2, natural inhibitor	Mouse	(84), 1988
	Purified and fragmented acrosin	Porcine	(85), 1990
	Purified acrosin, labeled ZP	Porcine	(86) (87), 1991
	Recombinant acrosin, labeled ZP	Porcine	(88), 1995 (89), 1998
		Rabbit	(90), 1996
	ā-Acrosin mAb, fucoidan	Human	(91), 1998
	Sperm crude extract from various species, labeled ZP	Porcine, Bovine, Ram, Hamster, Mouse, Rat	(92), 1993
	Sperm crude extract, labeled ZP	Porcine	(93), 1994
Primary binding	Synthetic and natural trypsin inhibitors, IVF assay	Mouse	(63), 1981
Sperm-egg fusion	Synthetic and natural trypsin inhibitors, IVF assay	Mouse	(94), 1976
		Hamster	(95), 1993

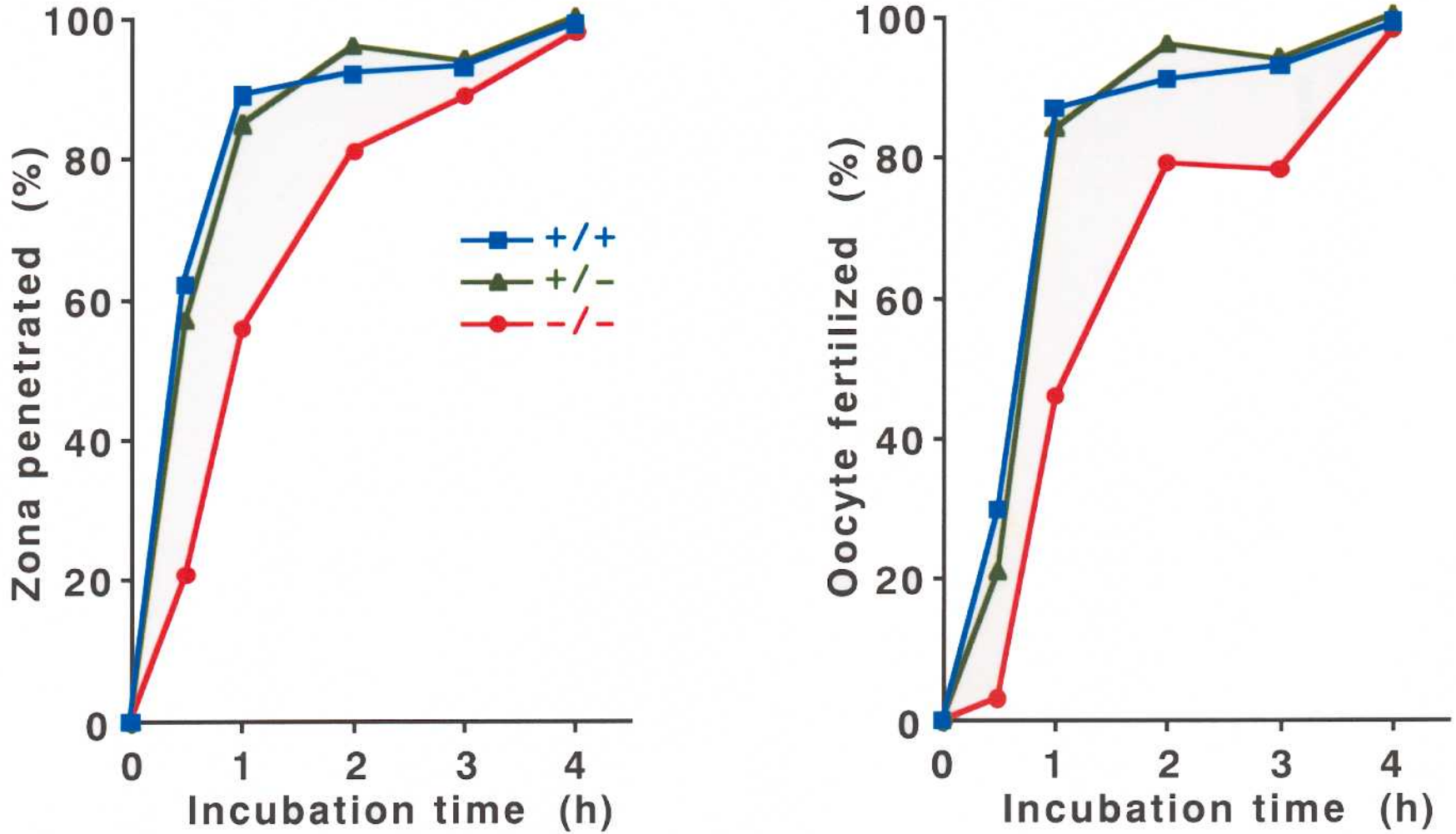

Fig. 4. ***In vitro*** **fertilization using wild-type, heterozygous, and homozygous mouse sperm for the targeted mutation in the acrosin gene.** Capacitated cauda epididymal sperm from the wild-type (blue square), heterozygous (green triangle), and homozygous (red circle) mice were incubated with cumulus-intact oocytes. This figure was reproduced by modifying the data of table 1 in Ref. 104.

in fertilization. In CHAPTER II, I have identified candidate protease(s) that may play an important role in sperm penetration of ZP. Moreover, the difference of acrosomal serine protease system among three rodent animals is discussed in CHAPTER III.