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Mini Review

Role of human prostasomes in the activation of spermatozoa

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Abstract

Prostasomes are small vesicles of prostatic origin contained in human semen. Their composition is peculiar under many aspects. Cholesterol is abundant and many proteins are endowed with enzymatic or other activities. The function of prostasomes has been amply debated and several hypotheses have been put forward. The liquefaction of semen, spermatozoa motility, antibacterial activity and immunological functions have been related to prostasomes. Under certain aspects, prostasomes resemble synaptosomes. The fusion of prostasomes to spermatozoa enriches spermatozoa with cholesterol and causes bursts of cytoplasmic sperm calcium. The interaction of spermatozoa and prostasomes should be limited to vagina since prostasomes are immobile and do not follow spermatozoa in the superior female genital tract. Calcium bursts would increase spermatozoa motility, where cholesterol would decapacitate spermatozoa, so preventing untimely activation. Since spermatozoa receive many different molecules from prostasomes, additional effects are also possible. Prostasomes makes spermatozoa more apt to be activated by progesterone in the proximity of the ovum. Therefore, the fusion between spermatozoa and prostasomes would influence spermatozoa behaviour under many aspects and might be relevant for fecundation. The richness of molecular species in prostasomes is amazing and these small vesicles are expected to lead to many more discoveries in the field of human reproduction.

Keywords: acrosomal reaction - cytosolic calcium - membrane fusion - prostasomes - semen - spermatozoa

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Prostasomes

Prostasomes are membranous vesicles secreted by the prostate gland. They were first discovered by Ronquist *et al*., in Sweden [1, 2] who also proposed for them the name of "prostasomes" because of their prostatic origin [1-7]. Prostasome-like particles have been found in the semen of other mammals [8-10]; however, their composition and amounts differ from those of human material. Moreover, different fecundation patterns (intravaginal vs intrauterine) may make these particles functionally dissimilar, depending on the species. The uncertainty of the production site is a major concern, when considering the prostasome-like particles contained in the semen of non-human mammals.

Prostasome morphology

Electron microscopy examination [11, 12] shows round particles surrounded by a membrane and containing electron-dense material. The size of prostasomes has been measured with various methods. Light scattering measurements [12, 13] confirmed microscopy data and revealed a population of particles with an average diameter of 150-200 nm.

Prostasome components

The lipid composition of prostasomes is peculiar; cholesterol is present in high amounts as is sphingomyelin, whereas phosphatidylcholine is less abundant [11, 12]. Therefore, these membranes differ amply from sperm plasma membranes [14, 15] that contain less sphingomyelin and more phosphatidylcholine with a cholesterol:phospholipid ratio of 0.83 [16]. The cholesterol to lipid phosphorus ratio in human prostasomes is about 2 [12]; this may be interesting since cholesterol may have roles in sperm capacitation [17]. Morever, prostasome lipid are rich in saturated fatty acid.

Prostasomes contain many small molecules and ions (Ca^{2+}, Zn^{2+}, GDP, ADP and ATP) and a number of enzymes among which phopholipase A, ATPase and peptidases [18-23], to list only few. Many prostasomal proteins have been recently identified through the analysis of proteome by Utleg *et al*. [24]. These authors found 139 proteins; enzymes account for 33.8% of total, transport structural proteins for 19.4%, chaperone proteins for 5.8%, GTP proteins for 14.4% and signal transduction proteins for 17.3%. Non-identified proteins were 9.4%.

Human semen is rich in prostasomes; ratio prostasome protein/spermatozoa protein is about 2. The presence of neuroendocrine markers such as chromogranin B, neuropeptide Y and vasoactive intestinal polypeptide [7] depicts prostasomes as neuroendocrine-like vesicles.

Physiological roles of prostasomes

Although prostasomes are small particles, they contain many different molecules. Moreover, several prostasomal components are found in other tissues and cell types where they exert known physiological roles. It is, therefore, reasonable to raise the question of their meaning in prostasomes.

The physiological significance of human prostasomes has been long debated and various possibilities have been put forward: among them, the enhancement of sperm motility [25], the liquefaction of semen [26] and immunosuppression [27-31]. The motility of spermatozoa is enhanced by prostasomes [25, 32-37] and antibacterial prop-

Table 1 Lipid content of human prostasomes.

Cholesterol (a)	08 ± 01
Total lipid phosphorus (a)	04 ± 0.1
Cholesterol/Phospholipid ratio	2.0 ± 0.8
Phosphatidylethanolamine (b)	15 ± 4
Phosphatidylcholine (b)	12 ± 3
Sphingomyelin (b)	53 ± 12
Phosphatidylserine	14 ± 3
Phosphatidylinositol	6 ± 3

(a) Results are expressed as $\mu mol.mg^{-1}$ protein ± SE
(b) Results are expressed as percentage of total lipid phosphorus in each phospholipid class ± SE

erties of prostasomes have been described [38, 39], including an antioxidant capacity [see ref. 40].

Prostasome to spermatozoa fusion

It has been reported that prostasomes interact [41] or even fuse [42] with spermatozoa. Interaction and/or fusion between spermatozoa and prostasomes may help throw some light on the role of prostasomes.

A fusion between prostasomes and spermatozoa has first been described by Arienti and coworkers [42] who used a lipophilic fluorescent derivative of rhodamine (octadecyl rhodamine G; R_{18}) to detect the movement of lipid from a membrane population to another [43]. The extent of fusion can be expressed as percentage, taking as 100% the theoretical maximal lipid mixing [44].

The fusion of prostasomes and spermatozoa is a pH and protein-dependent phenomenon (Fig. 2, 3). It increases with decreasing of the pH values of prostasome/spermatozoa mixtures and it is suppressed by the simultaneous destruction of prostasome and spermatozoa proteins. Usually, the fusion is investigated at pH 5.0, but it is still detectable at pH as high as 6 or 7. Yet, it is absent at pH 8. Since the average pH value of semen is around 7.6 [45] small variations of pH may trigger the fusion process. Moreover, in the so-called split ejaculation, spermatozoa appear to be emitted with the first fractions, together with the prostatic secretions, more acidic than the bulk of semen [46].

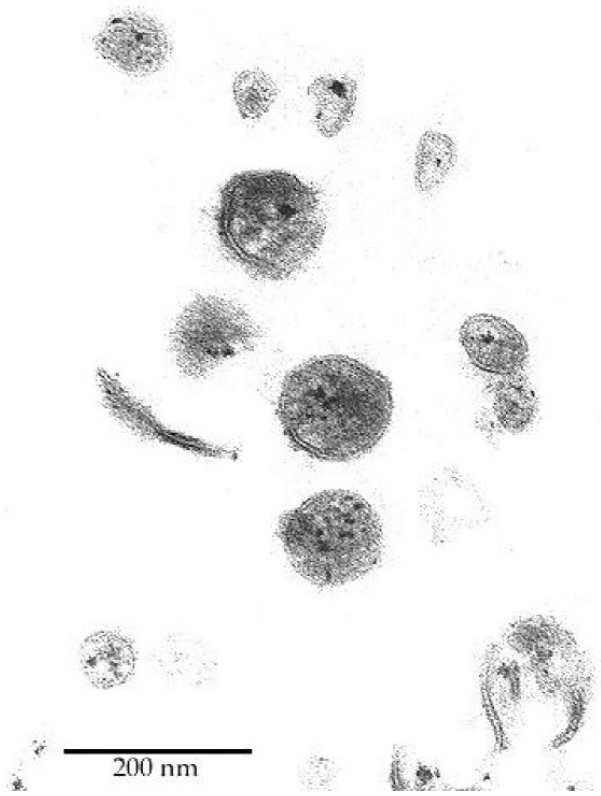

Fig. 1 Transmission electron micrograph of human semen prostasome vesicles.

The fusion between prostasomes and spermatozoa is never very large (10-20%), even in best conditions, but it is a quick phenomenon with a hyperbolic time course; it is practically finished 10 min after mixing prostasomes and spermato-

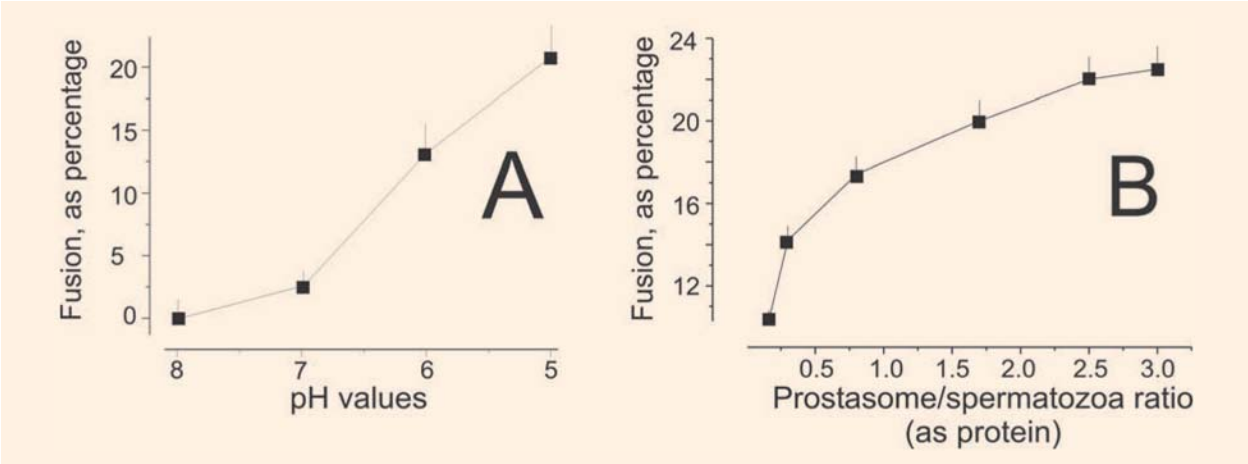

Fig. 2 Fusion of spermatozoa and prostasomes. (A) The effect of pH; (B) Effect of prostasome to spermatozoa ratio.

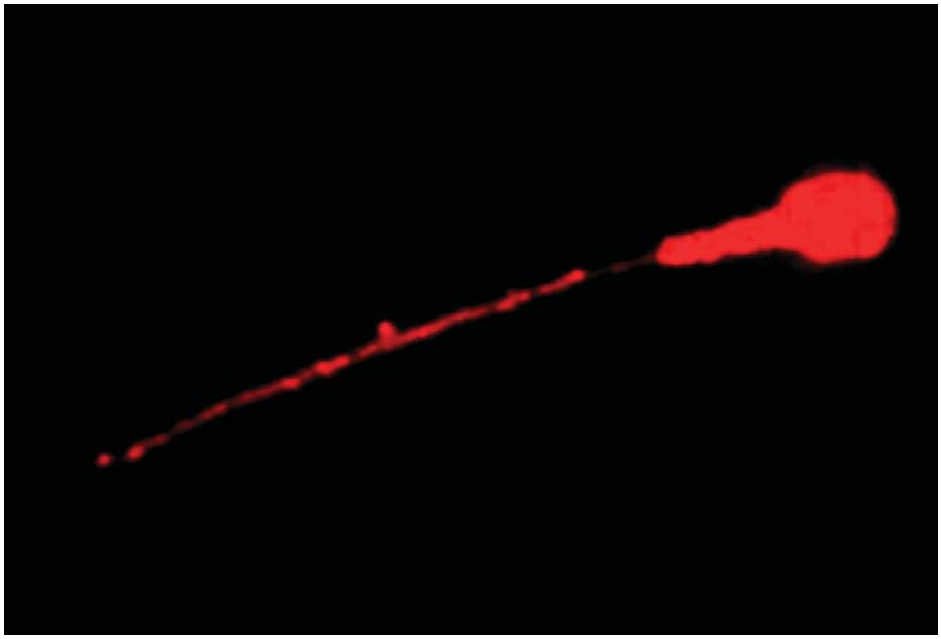

Fig. 3 Appearance of spermatozoa after fusion with R_{18}-loaded prostasomes. Spermatozoa were mixed for 110 min at pH 5.0 with R_{18}-labelled prostasomes. Magnification x 1000.

zoa [42] (Fig. 4). On the other hand, its quickness may have a physiological relevance, considering that the time of contact of prostasomes and spermatozoa at pH values low enough (below 7.0-7.5) to produce fusion is probably rather limited in physiological conditions.

Lipid and membrane-bound protein are both transferred through fusion [47-49] and some characteristics of sperm membranes, such as fluidity, change upon fusion with prostasomes [15]. The fusion appears to be specific for spermatozoa because it does not occur with lymphocytes [50], probably due to the necessary proteins, not yet identified. Other cell types have not been tested.

Spermatozoa cytosolic calcium

The fusion process may transfer all molecule types and ions contained in prostasomes besides lipid and protein. A particular attention has been given to calcium because it is contained in prostasomes [51] and is important for sperm motility and capacitation [52-54]. During the fusion process, calcium is released to spermatozoa as revealed by the increase of sperm $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$ is a transient process since the ion exchange readily and rapidly with extracellular Na^+. Upon omitting Na^+ from the external milieu, the increase of $[Ca^{2+}]_i$, parallels the extent of fusion as measured with R_{18} [55]. The bursts of

Table 2 Possible steps in spermatozoa activation.

Site	Event	Effect	Physiological meaning
Vagina	Spermatozoa/ prostasome fusion	↑ $[Ca^{2+}]_i$ ↑ Cholesterol Spermatozoa acquire a number of lipid and of protein	Increased motility Decreased ability to capacitate Long lasting effects
Uterus and tubae	Migration of spermatozoa	↓ Cholesterol	Spermatozoa acquire ability to capacitate
Near the ovum	Presence of progesterone	↑ $[Ca^{2+}]_i$ Acrosome reaction	Spermatozoa fused with prostasomes react better

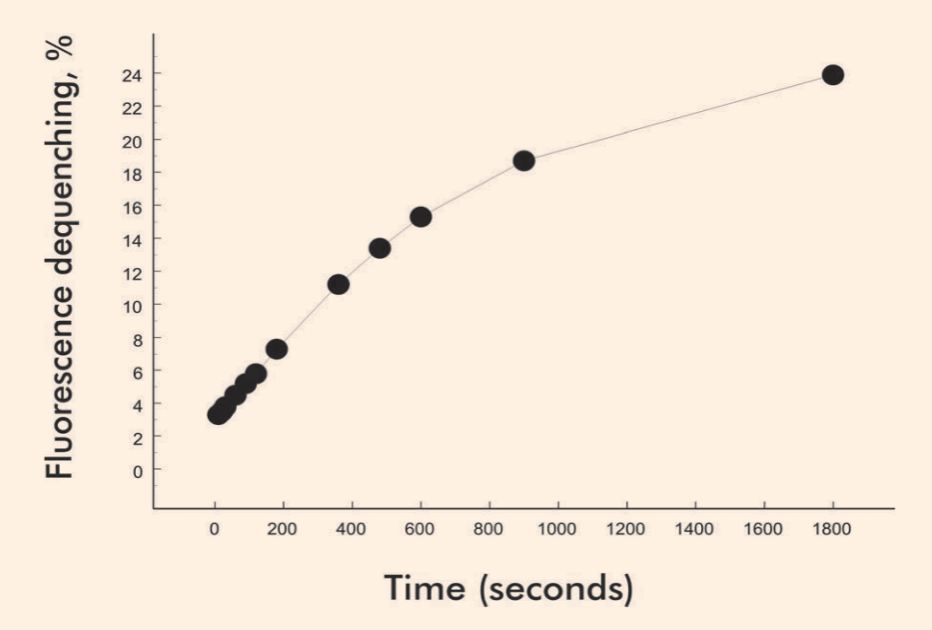

Fig. 4 Time-course of the fusion process. Spermatozoa were mixed with R_{18}-labelled prostasomes at pH 5.

spermatozoa $[Ca^{2+}]_i$ produced by the fusion with prostasomes appear to be physiologically in contrast with the delivery of cholesterol, since this lipid decreases the activation of sperm [17, 56-59], where calcium increases it. The simultaneous delivery of cholesterol and calcium, should activate the spermatozoa, increasing their motility (effect of $[Ca^{2+}]_i$) [37], although not permitting the occurrence of the acrosome reaction (effect of cholesterol). This fact would be interesting because the fusion with prostasomes must occur in vagina, whereas the acrosome reaction is a phenomenon taking place in the proximity of the oocyte.

Spermatozoa capacitation and acrosome reaction

Ejaculated human spermatozoa must be capacitated and undergo the acrosome reaction before fertilizing the oocyte [60]. The molecular mechanisms through which this is accomplished have amply been discussed. Attention has been given to the role of cholesterol to phospholipid ratio in sperm membranes [56, 57, 59, 61-63]. The loss of cholesterol during the migration of spermatozoa toward the ovum may be an activating factor.

The discovery that progesterone may act on spermatozoa through a mechanism that does not require a genomic pathway has thrown some more light on the activation of spermatozoa [64]. The effects of progesterone are mediated essentially by the increase of $[Ca^{2+}]_i$, the stimulation of the activity of phospholipases, the phosphorylation of proteins and efflux of chloride [65-68].

During fusion with prostasomes, lipids, proteins and ions [47-49, 55] can be delivered to spermatozoa. This is likely to further influence the behaviour of spermatozoa and their response to activating or inhibiting agents for some time after the fusion process.

The acrosome reaction is a necessary step in the sperm maturation. Before the passage through the epididymis, spermatozoa are unable to undergo the acrosomal reaction, but they have acquired this capacity when they reach the cauda epidydimis. Yet, spermatozoa are not allowed to acrosome-react, until they arrive in the proximity of the ovum. Therefore the cells maintain a sort of unstable equilibrium that allows them to acrosome react in the upper female genital tract, but not before.

The interaction with zona pellucida signals sperm to undergo the acrosome reaction [60] that can be initiated on capacitated spermatozoa following the activation of extragenomic progesterone receptors [65]. Progesterone also increases spermatozoa $[Ca^{2+}]_i$ and its effects are independent and additive to those produced by the fusion with prostasome [69].

In vagina, spermatozoa may acquire prostasomal components that would, directly or indirectly, modulate the acrosome reaction that takes place in the proximity of the ovum. Progesterone (1 μM at 37°C and in 30 min) does not induce the acrosome reaction [70], although spermatozoa cytosolic calcium increases in these conditions [69]. Usually, progesterone is required in concentrations about 10-15 μM for several hours to induce the acrosome reaction [59, 71, 72].

Previously fused spermatozoa were stimulated with 1 μM progesterone and the increase of sperm cytosolic calcium was larger than that due either to fusion or to progesterone alone [69]. Therefore progesterone and fusion show an independent and additive effect. The acrosome reaction was studied through the FITC-labeled pisum sativum agglutinin [73]. Fused spermatozoa underwent acrosome reaction better than the non-fused cells. This is in agreement with the finding the fused spermatozoa show a larger increase of $[Ca^{2+}]_i$ in the presence of low doses of progesterone [69].

Therefore, the fusion of prostasomes to spermatozoa, occurring in vagina, could alter the properties of spermatozoa producing several effects: (a) $[Ca^{2+}]_i$ bursts (stimulating spermatozoa movement); (b) delivery of cholesterol to spermatozoa (prevents an untimely acrosome reaction and (c) increased response to progesterone (calcium increase and improved acrosome reaction).

Conclusions

Since the time of their discovery, prostasomes, small particles contained in human semen that, since the time of their discovery, have revealed interesting properties. One of the most discussed points is their physiological relevance in human reproductive physiology. Some hypotheses have been put forward, but it is reasonable to think that future research will reveal much on this subject.

It is possible that the contact of spermatozoa with prostasomes may have a large part in spermatozoa maturation. The fusion of prostasomes and spermatozoa may help to keep spermatozoa in the delicate equilibrium involved in capacitation and acrosome reaction processes.

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