CHAPTER-I

Role of HABP1 in sperm function
4.1 INTRODUCTION

Presence of HABP1 in spermatozoa and its functional importance in sperm-oocyte interaction has been demonstrated earlier (Ranganathan et al., 1994 & Bronson et al., 1998). HABP1 has been reported to be a phosphoprotein and its phosphorylation in the sperm increases under Hyaluronan (HA) stimulation, suggesting its involvement in HA mediated cellular signaling (Ranganathan et al., 1995). To substantiate the earlier observations, a study on the effect of anti-HABP1 antibody and HA on sperm motility was conducted. The role of HABP1 in acrosome reaction was also further investigated in the present study. Its localization in the epididymis too was aimed at in order to understand the expression and function of HABP1 in the spermatozoa.

4.2 RESULTS

4.2.1 Effect of antibodies against HABP1 and hyaluronic acid on human sperm motility

Motility of human sperms incubated with various agents was recorded at various time intervals and the different kinematic parameters were analyzed using CASA (Computer-Aided Sperm Analysis). For the sperm motility studies with anti-tHABP1 antibody, IgG was purified from anti-tHABP1 antiserum using protein A column and used at dilutions of 1:50 and 1:20. Normal Rabbit IgG at the same dilution was used as a control. Various kinematic parameters were plotted against incubation time (in h) [VAP- Fig. 4A; VSL - Fig. 4B; VCL- Fig. 4C; % MOTILITY- Fig. 4D; ALH- Fig. 5A; BCF- Fig. 5B; STR- Fig. 5C; LIN - Fig. 5D; WOB - Fig. 5E]. As shown in Fig. 4D, only the percentage motility of the sperms was affected at 22 h of incubation. The percentage motility of sperms in the presence of anti-tHABP1 IgG, both 1:20 (light blue triangles) and 1:50 (dark blue squares) was significantly higher than their respective controls of Rabbit IgG (1:20 and 1:50), p≤0.001. ALH and BCF parameters also showed a change in the anti-tHABP1 IgG treated samples when compared to their control, Rabbit IgG. At 6h, ALH of anti-tHABP1 IgG treated samples showed a lower value (Fig. 5A, magenta diamonds) at p≤0.04, when compared to its control Rabbit IgG [Fig. 5A, 1:20 (light blue
Figure 4.

KINEMATICS OF HUMAN SPERM: EFFECT OF ANTI-HA-BINDING PROTEIN ANTIBODIES ON VAP, VSL, VCL AND PERCENTAGE MOTILITY

* : Significantly higher at 22 h than its control, Rabbit IgG ( ), 1:50, p ≤ 0.001

* : Significantly higher at 22 h than its control, Rabbit IgG ( ), 1:20, p ≤ 0.001
Figure 5.

KINEMATICS OF HUMAN SPERM: EFFECT OF ANTI-HA-BINDING PROTEIN ANTIBodies ON ALH, BCF, STR, LIN AND WOB

* : Significantly lower at 6 h than its control, Rabbit IgG (■ ), 1:20, p ≤ 0.04

△: Significantly higher at 22h than its control, Rabbit IgG(■), 1:50, p ≤ 0.02
triangles]) and BCF of anti-tHABP1 IgG treated sample [Fig. 5B, 1:50 (red triangles)] showed a higher value at 22 h when compared to its control, Rabbit IgG [Fig. 5B, 1:50 (dark blue squares)], p≤0.02.

HA polymer and its controls, the HA disaccharide and HA monosaccharides (N-Acetyl glucosamine and D-glucuronic acid) did not have at any effect, at any concentration on any kinematic parameter, at any observed time of incubation (Figs. 6A-D and Figs. 7A-E).

4.2.2 Release of phosphorylated form of HABP1 during acrosome reaction

Rat sperms after initial incubation in BWW medium for the stipulated 30 min, were stimulated with progesterone for the induction of acrosome reaction and then further incubated for 15 min. After incubation, an aliquot of the cells was stained with trypan blue to check cell viability. After that, the cells were centrifuged and the supernatant that contained the proteins released from the sperms during capacitation and after acrosome reaction, was collected. Supernatant (60 μl) from each of the samples was taken, dot blotted and processed for the detection of HABP1 in the medium using anti-tHABP1 antibody. As shown in Fig. 8A, the concentration of HABP1 in the medium progressively increased from the control medium (slot 2) to the medium of acrosome reacted sperms (slot 5). The medium of non-capacitated (slot 3) and capacitated sperms (slot 4) also showed the presence of HABP1. Since BSA was the major component of the medium (3 mg/ml), 30 μg BSA was used as a control (slot 6) and the signal seen was found to be equivalent to the background signal of the control media, seen in slot 2, suggesting a release of HABP1 during acrosome reaction.

In order to learn if HABP1 is released from the acrosome reacted sperm in a phosphorylated form, sperms were labeled in the presence of γ-[ATP] for 20 min and then stimulated with progesterone (5 μg/ml) and HA (250 μg/ml) for another 15 min. After that, the medium was immunoprecipitated using anti-tHABP1 antibody, to detect the presence of HABP1. The cell pellet too was lysed and subjected to immunoprecipitation to serve as a control for proper in vivo cell labeling. Fig. 8B shows the CBB stained SDS-PAGE gel, where the IgG heavy chain at 50 kDa was
Figure 6.

KINEMATICS OF HUMAN SPERM: EFFECT OF SUGARS ON VAP, VSL, VCL AND PERCENTAGE MOTILITY

![Graphs showing kinematics of human sperm](image-url)
Figure 7.

KINEMATICS OF HUMAN SPERM: EFFECT OF SUGARS ON ALH, BCF, STR, LIN AND WOB

A  
ALH (μm)  
0 5 10 15 20 25  
Time of Incubation (h)

B  
BCF (Hz)  
0 5 10 15 20 25  
Time of Incubation (h)

C  
STR (%)  
0 5 10 15 20 25  
Time of Incubation (h)

D  
LIN (%)  
0 5 10 15 20 25  
Time of Incubation (h)

E  
WOB (%)  
0 5 10 15 20 25  
Time of Incubation (h)

- Control  
- HA polymer- 50μg/ml  
- HA monosaccharide- 50 μg/ml  
- HA disaccharide- 50μg/ml  
- HA disaccharide- 150μg/ml  
- HA monosaccharide- 150μg/ml  
- HA polymer -150μg/ml
Fig. 8A. RELEASE OF HABPI FROM RAT SPERM AFTER ACROSOME REACTION. 60 µl BW medium in which the sperms were acrosome reacted was taken along with controls and dot-blotted on an NC membrane and probed with anti-tHABPI antibody, α-Rabbit IgG-AP conjugate and NBT/BCIP color system. The samples are as follows: slot 2 - only medium with BSA and without sperms, slot 3 - medium with sperms but no BSA; slot 4 - medium with both sperms and BSA and slot 5 - medium with sperm BSA and progesterone (5µg/ml). Slot 1 with 2 µg tHABPI as a positive control and slot 6 with 30 µg BSA as the negative control for the dot blot analysis are also shown.

Fig. 8B-C. RELEASE OF PHOSPHORYLATED FORM OF HABPI FROM RAT SPERMS AFTER ACROSOME REACTION. Rat sperm lysate and sperm incubation media after induction of acrosome reaction were subjected to immunoprecipitation using anti-tHABPI antibody. The CBB stained SDS-PAGE gel is seen in Fig. 8B. The control for equal loading; the IgG heavy chain is seen at ~53 kDa (lanes 2-11). BSA has been used as a marker (lane 1), besides the LMW marker used (lane 12). Fig. 8C shows the autoradiogram of the SDS-PAGE gel. Immunoprecipitated samples from the media with BSA but without sperms (lane 2), with sperms but no BSA (lane 3), with sperms and BSA (lane 4), with sperms with BSA and stimulated with progesterone (lanes 5) and HA (lane 6) were run and exposed for autoradiography. The cells lysates of sperms without BSA (lane 8), with BSA (lane 9), with BSA and progesterone (lane 10) and with BSA and HA (lane 11) are also shown. Lane 7 shows medium with BSA alone.
used as a control for equal amount of loading in all lanes. The CCB stained gel was then autoradiographed. The autoradiogram is shown in Fig. 8C. As clearly evident, a 34 kDa phosphorylated HABP1 band was detected in the medium of capacitated sperms (lane 4), of progesterone treated (lane 5) and HA treated (lane 6) sperms, suggesting release of phosphorylated form of HABP1 during acrosome reaction. HABP1, probably is phosphorylated only during capacitation and acrosome reaction, since HABP1 detection was not seen in the medium of the non-capacitated (lane 3) sperms, although, HABP1 phosphoprotein was detected in the non-capacitated sperms (lane 8), reflecting that phosphorylation has taken place in non-capacitated cells. This data confirmed that HABP1 is released in the phosphorylated form only after capacitation.

4.2.3 Binding of HABP1 to mouse zona

Varying concentrations of solubilized mouse zona (0, 0.2, 0.5, 1.0, 2.0 and 3.0 μg) were slot blotted onto an NC membrane, probed with biotinylated rHABP1 and Avidin conjugated to AP and the color was developed with NBT/BCIP color system. Preliminary result (Fig. 9) obtained shows a concentration dependent binding of HABP1 to mouse ZP.

4.2.4 Immunohistochemical localization of the HABP1 in the caput and cauda epididymis of adult rats

Discrepancy between data of no considerable effect of anti-tHABP1 IgG and HA on sperm motility and the observation of HABP1 showing binding specifically to ZP and its release after acrosome reaction prompted us to look at the localization of the protein in the sperms of rat caput and cauda sperms. For this, immunohistochemistry was performed on rat caput (Figs. 10A-E) and cauda (Figs. 11A-E) epididymis sections. Figs. 10A-E show the immunohistochemical staining done with anti-tHABP1 and anti-rHABP1 antibodies. Normal rabbit serum and pre-immune serum served as controls for anti-tHABP1 and anti-rHABP1 antibodies, respectively. As shown in Fig. 10B, anti-tHABP1 antibody showed no staining in the sperms (‘sp’-sperms; ‘CD’-cytoplasmic droplet), but intensely stained the interstitium (‘Int’ Fig. 10A, arrow). Ep, which denotes the epithelium (Fig. 10B) was
Fig. 9. BINDING OF HABP1 WITH MOUSE ZP. Solubilized mouse ZP after protein estimation was slot-blotted and probed with biotinylated rHABP1, streptavidin conjugated to AP and NBT/BCIP color system. Signal obtained with varying concentration of ZP lysate [0 μg (slot 1), 0.2 μg (slot 2), 0.5 μg (slot 3), 1.0 μg (slot 4), 2.0 μg (slot 5) and 3.0 μg (slot 6)] is seen.
Fig. 10. EXPRESSION OF HABP1 IN RAT CAPUT EPIDIDYMIS. Rat caput epididymis sections were probed with anti-tHABP1 antibody, 1:50 (Panels A&B) and anti-rHABP1 antibody, 1:20 (Panel D). Normal rabbit serum, 1:50 (Panel C) and pre-immune serum, 1:250 (Panel E) controls are seen.
also not stained. In contrast to the staining of at least the interstitium by anti-tHABP1 antibody, no signal was detected with anti-rHABP1 antibody in the caput epididymis including the sperms (Fig. 10D). Normal rabbit serum (Fig. 10C) control was negative. Fig. 10E shows the pre-immune serum control, which too was expectedly negative.

Cauda epididymis also showed the same staining pattern as that seen for caput epididymis. Briefly, anti-tHABP1 antibody stained the interstitium (Fig. 11A) with no staining in the sperms (Fig. 11A & B). Anti-rHABP1 antibody did not stain any region of the cauda epididymis (Fig. 11D). Normal Rabbit serum (Fig. 11C) and pre-immune serum (Fig. 11E) controls were negative.

4.2.5 Presence of HABP1 in rat sperm lysate

It was important to probe the rat sperm lysate for the presence of HABP1, in order to support the acrosome reaction (Fig. 8) and zona binding (Fig. 9) data, since immunohistochemistry of caput and cauda epididymis (Fig. 10-11) and CASA analysis suggested an absence of HABP1 in the sperms.

Rat sperms after perfusion from the cauda epididymis were washed after passing through 5% Ficoll-PBS and the lysate was prepared by boiling the washed sperm pellets in 1 x Laemmli buffer.

As shown in Fig. 12, the presence of 34 kDa HABP1 (single arrowhead) was seen in the rat sperm lysate (lane 1). Purified rHABP1 was seen getting detected in lane 2. The additional 68 kDa protein was also detected in the rat sperm lysate (lane 1, double arrowhead). This confirmed the presence of HABP1 in rat sperm.

4.3 DISCUSSION

Computer-Aided Sperm Analysis (CASA) system is designed for objective assessment of the movement characteristics (kinematics) of spermatozoa after optimization. Kinematic parameters measured are abbreviated according to Boyers et al (1989) and defined as: VCL, curvilinear velocity [termed ‘track speed’ by HTM (Hamilton-Thorne system)]: the time-averaged velocity obtained from distances between two consecutive track points; VSL, straight-line velocity (termed ‘progressive velocity’ by HTM): the velocity obtained from the distance between the
Fig. 11. EXPRESSION OF HABP1 IN RAT CAUDA EPIDIDYMIS. Rat cauda epididymis sections were probed with anti-tHABP1 antibody, 1:50 (Panels A&B) and anti-rHABP1 antibody, 1:20 (Panel D). Normal rabbit serum, 1:50 (Panel C) and pre-immune serum, 1:250 (Panel E) controls can also be seen.
Anti-tissue HABP Ab, 1:50

A

Normal Rabbit Serum, 1:50

C

Preimmune Sera, 1:250

E

Anti-tissue HABP Ab, 1:50

B

Anti-recombinant HABP Ab, 1:250

D

Int

20µm

Sp

10µm

20µm

20µm
Fig. 12. PRESENCE OF HABP1 IN RAT SPERMATOZOA. Lysate from freshly isolated rat cauda sperms was prepared after washing the rat sperms with 5% Ficoll-PBS and resolved on a 12.5% SDS-PAGE and probed with anti-tHABP1 antibody and anti-rabbit IgG-HRP conjugate and developed with the ECL system. Lane 1 shows Ficoll washed rat sperm lysate and lane 2 shows purified tHABP1 (1 µg).
first and the last track points; VAP, average-path velocity: the time-averaged velocity obtained from smoothing the original path which joined up consecutive track points; ALH, amplitude of lateral displacement of the centroid: the width of centroid oscillation measured as twice the maximum value of the distance of any point on the original track from the corresponding 5-point average; LIN, linearity: \((VSL/VCL) \times 100\%\); STR, straightness (termed ‘linearity index’ by HTM): \((VSL/VAP) \times 100\%\). Present study reveals negligible effect of either hyaluronic acid upto the level of 150 \(\mu\)g/ml or the antibody against HABP1 on human sperm motility parameters during the early hours of incubation (3-6 h). Most significant effect seen was in percentage motility of the sperm at 22 h in the presence of anti-HABP1 antibodies (both 1:20 and 1:50 dilution). Effect seen mostly after 22 h of incubation suggests an increase in the surface expression of HABP1 in the sperm at this time of incubation, enabling the exogenously added agents in the medium to interact with HABP1 and exert their effect. This hypothesis is supported by the observation of Bronson et al (1998), who reported an increase in expression of HABP1/gC1q receptors on sperm surface during capacitation. Redistribution of protein occurring during capacitation is a common phenomena. Various reports exist on the surface distribution of sperm proteins like laminin & vitronectin (Fusi et al., 1992) and fibronectin (Fusi and Bronson, 1992). In support, Rochwerger and Cuasnico (1992) too describe a change in the expression pattern of a rat sperm epididymal glycoprotein DE of Mr 37 kDa after capacitation and correlate the change with the occurrence of acrosome reaction.

Our results show no effect of hyaluronic acid (50 and 150 \(\mu\)g/ml) or its controls at any point of time and at any concentration. It is possible that “under these experimental conditions”, no effect of HA and its controls on sperm motility is seen, although such effect has been reported to occur. Huszar et al (1990) and Sabracia et al (1997) reported that hyaluronic acid (200 and 250 \(\mu\)g/ml, respectively) as a sodium salt improves the velocity and retention of motility of freshly ejaculated human spermatozoa even after 30 h of incubation. Since the concentration used in this study (50 and 150 \(\mu\)g/ml) was much below that used by Sbracia et al (1997) and
Huszar et al (1990), it could be that ‘no effect’ was due to low concentration of HA used in these experiments. But, Perry et al. (1996) report that the use of Healonid, a preparation having a very high concentration of HA (10 mg/ml) as sodium hyaluronate, did not have any significant effect on sperm motility either directly after isolation of spermatozoa from sperm (0 h) or during incubation (3 & 6 h). This report, thus rules out the possibility of the effect being in any way concentration dependent and supports our observation of seeing “no effect“ of HA and its controls on sperm motility. HA-motility relationship can be, however, also discussed in terms of protein phosphorylation. From our laboratory, Ranganathan et al (1995) has shown that increase in total protein and specifically tyrosine phosphorylation in sperm occurs after HA stimulation of sperms and tyrosine phosphorylation has been implicated to have an effect on sperm motility (Hayashi et al., 1987; Morisawa and Hayashi, 1985). Thus, it may be speculated that HA has an effect on sperm motility through tyrosine phosphorylation, although the present information does not seem to support it.

Shedding off of the sperm proteins results after acrosome reaction. Ranganathan (1995) demonstrated that after acrosome reaction the expression of HABP1 on the sperm surface decreased suggesting HABP1 release during acrosome reaction. One way of looking at the fate of a sperm surface protein during acrosome reaction is to look at its expression on the sperm surface, while another way is to see its presence in the medium, in which the sperms acrosome react, provided the protein is actually released from the sperm. The latter approach was the one we followed since the former one had already been carried out.

Accordingly, in this study we looked for the presence of HABP1 in the medium of acrosome reacted sperm by immunoprecipitation. Detection of HABP1 in the medium of acrosome reacted sperms substantiates the earlier data of release of HABP1 from sperm surface after acrosome reaction (Ranganathan, 1995). Similar observations were made by Fusi et al (1994) in regard to the release of vitronectin from the human spermatozoa after acrosome reaction, as detected by immunoblotting.
with anti-vitronectin antibody of the supernatant in which sperms was acrosome reacted.

Release of HABP1 in the phosphorylated form during capacitation and acrosome reaction was observed. Release of HABP1 as a phosphoprotein supports the earlier observation of enhanced HABP1 phosphorylation under progesterone stimulation (Bharadwaj, 1995). Reports on increasing tyrosine phosphorylation under progesterone stimulation in sperm (Baldi et al., 1995; Luconi et al., 1995) exist. But, since HABP1 is a serine/threonine phosphoprotein, the fact that serine/threonine kinases are regulated by tyrosine phosphorylation (Anderson et al., 1990; Avruch et al., 1994) seems to be the only explanation for the increase in HABP1 phosphorylation (a threonine phosphoprotein) under progesterone stimulation.

Another interesting observation is the release of HABP1 from the sperm after stimulation with HA. HA has been shown to stimulate phosphorylation of sperm proteins including HABP1 (Ranganathan et al., 1995). HA has been shown to stimulate capacitation under in vitro conditions (Handrow, 1982) along with some recent reports which show that HA also induces acrosome reaction. vandeVoort et al (1997) reported that HA (100 \( \mu \)g/ml) enhances zona pellucida - induced acrosome reaction of Macaque sperm. They reported that the enhancement was observed only when HA exposure preceded sperm-zona binding, suggesting that HA interacts with the sperm surface, possibly via a receptor at the time of initiation of acrosome reaction. HA concentration used in our study was 250 \( \mu \)g/ml and this dosage is much higher than the dosage in the study by vandeVoort et al (1997) and this increase could actually result in induction of acrosome reaction rather than just enhancing the effect of zona-induced acrosome reaction. Psalti et al (1993) and Lenz et al (1983) too reported the effect of HA on acrosome reaction, along with Meizel and Turner (1986) who showed that HA stimulated acrosome reaction of capacitated hamster sperm.

The only factor, which possibly may raise doubts, is the use of [AT\(^{32}\)P] and the question, as to how it enters the cell. ATP due to high negative charge cannot
enter the cell and this limits its uptake by the cell. However, the possibility that proteins are released after acrosome reaction in a non-phosphorylated form and undergo \textit{in vitro} phosphorylation cannot be ruled out. The other possibility which exists is that the ectoprotein kinases present on the sperm surface which phosphorylate the sperm surface proteins, may phosphorylate HABP1 too. Ectoprotein kinases have been reported in the spermatozoa of various species, namely, rat (Atherton et al., 1985), human (Schoff et al., 1982; Pariset et al., 1983) and goat (Dey and Majumder, 1989). Sperm surface localization of HABP1 (Ranganathan et al., 1994) and its enhanced expression during capacitation (Bronson et al., 1998) strongly supports this hypothesis, wherein the surface HABP1 may be a substrate for ectoprotein kinases. Zhu et al (1997) report the phosphorylation of Osteopontin and bone sialoprotein by ectoprotein kinase activity utilizing \(\gamma-[^{32}\text{P}]\) and also demonstrate that a kinase with characteristics similar to Casein kinase II may be responsible for this kind of phosphorylation utilizing \(\gamma-[^{32}\text{P}]\). HABP1 too has five Casein kinase II phosphorylation sites (Deb and Datta, 1996) and thus, it is likely that the HABP1 on the sperm surface is phosphorylated by Casein kinase II like kinase present on the sperm surface. Also, interestingly, they report of a 34 kDa protein which gets phosphorylated by this casein kinase II like kinase. Zhu et al (1997) also demonstrate that the exogenously added Osteopontin and bone sialoprotein, are phosphorylated by ectoprotein kinase. This suggests a remote possibility of this happening with HABP1 released during acrosome reaction, wherein HABP1 is released after acrosome reaction in an unphosphorylated condition, and gets phosphorylated in the medium in the presence of the exogenous \(\gamma-[^{32}\text{P}]\) and the ectoprotein kinase present on the sperm surface. However, the release of unphosphorylated form of HABP1 from the sperm after acrosome reaction stimulated by progesterone, especially is ruled out since enhanced phosphorylation of HABP1 under progesterone stimulation is already demonstrated (Bharadwaj, 1995). It has been also ruled out by Zhu et al (1997) that the cell surface ATPases (Majumder and Biswas, 1979) hydrolyze ATP to generate free \(^{32}\text{P}\), and it is the free
[\textsuperscript{32}P] which then enters the cell and phosphorylate the proteins, since the short labeling time is not enough for this whole procedure to take place.

Ranganathan et al (1994) demonstrated that sperms pre-treated with anti-HABP1 antibodies did not bind to zona intact mouse eggs. This suggested that HABP1 might be involved in primary binding to zona. Concentration dependent binding of HABP1 to solubilized mouse ZP seen in this study, substantiates the observation of HABP1- ZP binding. Silver staining profile of the ZP lysate, revealed a 83 kDa band corresponding to mouse ZP3, suggesting the ZP3 to be the major component of the ZP lysate prepared. Thus, it could be understood that HABP1 binds to ZP3 present in the ZP lysate and is thus, involved in primary binding of sperm to the zona. From our laboratory, Kumar (1999) reported specific binding of HABP1 to the neoglycoconjugate DMA (D-mannosylated Albumin) or BSA-Mannose, as it is commonly referred to. Kumar (1999) also demonstrated the binding of HABP1 to human zona pellucida, which was abolished by competition with 20-fold excess of DMA or unlabelled HABP1, indicating that the binding of HABP1 to zona is through mannose or to be precise, clustered mannose. DMA has been reported to mimic the mannose oligosaccharide residues on the zona protein ZP3 (Mori et al., 1989; Benoff et al., 1993a and 1997b).

Importance of carbohydrate binding proteins in mammalian fertilization has been established (Tulsiani et al., 1997; Topfer-Peterson et al., 1995). Tulsiani et al (1992) reported the presence of high mannose/hybrid oligosaccharide chain(s) on the mouse ZP3, suggesting that the carbohydrate moiety(ies) of the ZP are likely to be the sperm receptor sites. Chen et al (1995) too reported the expression of mannose binding sites on human spermatozoa and established its role in sperm-zona pellucida binding. Reports on D-mannose inhibiting sperm penetration through zona in human (Mori et al., 1989) and rat (Shalgi et al., 1986) exist.

The observation that HABP1 binds to ZP also seems to be important from the physiological point of view because it suggests that HABP1 may have a role to play in zona-induced acrosome reaction. ZP3 acting as a sperm receptor for acrosome intact spermatozoa, through O-linked chains (Florman and Wassarman, 1985) and
triggering the acrosome reaction of bound spermatozoa has been demonstrated. Barratt et al (1994) reported that glycosylation was necessary in human recombinant ZP3 to induce the human acrosome reaction along with a report by Loeser and Tulsiani (1999), who discuss the role of carbohydrates in the induction of acrosome reaction in mouse spermatozoa. Additionally, reports exist which say that mannose ligand binding induces an aggregation of sperm surface mannose receptor, their relocation to the equatorial post acrosome region of the sperm head and the acrosome reaction (Benoff et al. 1993a, b, 1995, 1996a, 1997a).

Benoff et al (1993a, b & 1996a, b) also reported that the percentage of acrosome intact spermatozoa expressing surface mannose receptors is a marker for zona-binding potential in in vitro fertilization. Thus, the binding of HABP1 present in acrosome intact spermatozoa to ZP and its release after the acrosome reaction, suggests a role of HABP1 in zona induced acrosome reaction. Even, in terms of phosphorylation, reports exist on zona stimulated sperm phosphorylation prior to acrosome reaction (Saling et al. 1990; Leyton and Saling, 1989a, b; Beilfeld et al., 1994), which further support HABP1 phosphorylation during acrosome reaction. Thus, conclusively this study strongly indicates an important role of HABP1 in zona-induced acrosome reaction.

Immunohistochemically, it has not been possible to localize HABP1 in the sperms. However, it was confirmed by immunoblot analysis that HABP1 is present in the rat sperm. The rat sperms were washed by passing through Ficoll, in order to get rid of any component of the epididymal fluid sticking onto the sperm surface. It is, however, quite likely that the sensitivity of immunohistochemistry may not be enough to detect the levels of HABP1 in the sperm, although when a lysate is prepared from the same sperms the protein was detected. Alternatively, the epitopes may be masked, making the detection with the antibody difficult. Toshimori et al (1992) report that an epitope of a 54 kDa sperm surface sialoglycoprotein on the flagellum is masked by sialic acid residues, which they term as the "hidden determinant or cryptodeterminant". Moreover, it is possible that HABP1 has a
subsurface localization in the epididymis and only surfaces up after capacitation as seen by Bronson et al (1998).

Summarily, this study thus, suggests a capacitation dependent surface expression of HABP1 along with an involvement of HABP1 phosphoprotein in zona-induced acrosome reaction.