

## Proteins implicated in sperm capacitation

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Spermatozoa after being deposited in the female reproductive tract spend a considerable time in this foreign environment prior to fertilization of the oocyte. Chang and Austin independently observed<sup>1,2</sup> that this time spent by the spermatozoa in the female tract is not consequential but a necessary event in the life cycle of the male gamete, and Austin<sup>2</sup> first called this maturation period of spermatozoa as 'Capacitation'. Ever since, attempts have been made to understand and unravel the molecular mechanism of capacitation. Based on the results obtained so far, it is clear that capacitation is guided by novel signal transduction pathways influencing varied aspects of spermatozoa. Capacitation could be, thus, defined as the cumulative molecular, cellular and physiological changes that occur in spermatozoa in the female reproductive tract to achieve the final competence to fertilize the oocyte. This review is structured so as to first understand the key features of capacitation and then to survey the players which bring about these changes during capacitation.

**Keywords:** Capacitation, Protein implication, Sperm

### Features of capacitation

*Hyperactivation*—Yanagimachi<sup>3,4</sup> has observed that as the spermatozoa gain the ability to fertilize oocytes *in vitro*, their flagella starts beating more vigorously than before, thus heralding the discovery of "hyperactivation" which is now considered as a hallmark of capacitation. Hyperactivation is considered to be critical to the success of fertilization as it enhances the ability of the spermatozoa to traverse viscoelastic zones in the female reproductive tract more effectively than non-hyperactivated ones or to penetrate mucous substances of the cumulus oophorus and zona pellucida of the oocyte<sup>5,6</sup>. Hyperactivated spermatozoa can now be discriminated more objectively from the non-hyperactivated population during capacitation due to distinct changes in their motility kinematics using a computer aided semen analysis system<sup>7-9</sup>.

Hyperactivation is an energy driven process and the existing literature about the involvement of mitochondrial ATP synthesis in hyperactivation is very controversial. However, glycolysis has been found to be essential for generating ATP required for

hyperactivation in human<sup>10</sup> and rat<sup>11</sup> spermatozoa. Hamster<sup>12,13</sup> and mouse<sup>14</sup> spermatozoa do not acquire hyperactivation in the absence of calcium in the medium, clearly indicating a requirement for calcium during hyperactivation. Suarez *et al*<sup>15</sup> have observed that calcium levels rise specifically in the flagella of hamster spermatozoa<sup>15</sup> during hyperactivation. This increase in calcium during hyperactivation may occur due to the entry of calcium from extracellular sources through voltage gated calcium channels or due to intracellular release of calcium<sup>16</sup>, indicating the presence of internal calcium store(s) in spermatozoa. A less characterized sperm organelle, redundant nuclear envelope (RNE), has been proposed to be an intracellular store for calcium in bull spermatozoa and has also been implicated in hyperactivation<sup>17</sup>. It has been proposed for hamster spermatozoa that cyclic adenosine monophosphate (cAMP) might function by maintaining calcium at an optimum concentration inside the cell<sup>18</sup>. However, recently, in bovine spermatozoa it has been shown that calcium, and not cAMP, is required for hyperactivation<sup>19</sup>. The involvements of cAMP and bicarbonate ions in hyperactivation could be linked by the bicarbonate stimulated synthesis of cAMP, although no direct proof exists for this. It is, however, not clear if all these molecules directly influence hyperactivation or indirectly through some other means like stimulating capacitation dependent tyrosine phosphorylation.

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Although hyperactivation occurs at some point during capacitation, both phenomena have been found to be uncoupled in some cases, like spermatozoa from *tw32/+* mouse showing precocious hyperactivation while capacitation occurs on schedule<sup>20</sup>.

**Acrosome reaction**—Another hallmark of capacitation is the acrosome reaction in spermatozoa which is considered to be the end point of capacitation. During the acrosome reaction the outer acrosomal membrane of the spermatozoon fuses with the overlying plasma membrane and releases the acrosomal contents which facilitate penetration of the spermatozoa into oocyte<sup>21</sup>. The physiological significance of this reaction is further highlighted by the fact that males with spermatozoa lacking acrosome are reported to be infertile<sup>22</sup>. Breitbart<sup>23</sup> developed an *in vitro* system for elucidating the fusion mechanisms during acrosome reaction. Recently, it has been shown that actin polymerizes during capacitation and that the polymerized F-actin breaks down just before acrosome reaction<sup>24</sup>. The physiological trigger for acrosome reaction is either zona pellucida or progesterone<sup>25</sup> and can also be induced non-physiologically by calcium ionophore, A23187<sup>26</sup>, thus, implying an active role of the divalent cation in this event. Both extracellular and intracellular calcium sources are known to influence the acrosome reaction<sup>27</sup> and the effect may be mediated through its influence on calcium dependent molecules like protein kinase C, phospholipase C and calmodulin, which have been demonstrated to be involved in acrosome reaction (as reviewed<sup>28</sup>). However, the interdependence of one molecule on the other is not yet clearly worked out in spermatozoa as in other cells.

### Molecular basis of capacitation

It is over 50 years now since the phenomenon of capacitation was discovered, yet, the molecular basis of capacitation is not clearly established. Early on when acrosome reaction was considered as the end-point of capacitation, it was observed in rabbit that the removal of spermatozoa-bound seminal plasma components by the follicular fluid in the female reproductive tract was a prerequisite for the induction of acrosome reaction<sup>29</sup>, indicating that the nature has selected ways to arrest capacitation in the male reproductive tract and trigger it in the female reproductive tract. Eventually, it was demonstrated

that protein tyrosine phosphorylation is the backbone of the signal transduction mechanism of capacitation, which is triggered by cholesterol efflux. Therefore, the molecular basis of capacitation can be best understood by dissecting it into an event of cholesterol efflux, followed by the involvement of first and second messengers, and finally culminating in protein tyrosine phosphorylation of specific effectors of capacitation.

### Cholesterol efflux

Albumin was shown to trigger capacitation and it was also shown that the decapacitating capacity of seminal plasma lies with cholesterol bearing membrane vesicles present in rabbit and rat<sup>31,33</sup>. Davis has further shown that albumin triggers capacitation by reducing the cholesterol/phospholipid ratio of the sperm membrane. He has also demonstrated that uterine fluid is capable of causing substantial cholesterol efflux from the sperm membrane<sup>34</sup>. Eventually high density lipoproteins in the uterine and follicular fluid are presented as the native molecules in the above fluids, which can act as cholesterol acceptors and thus induce capacitation *in vivo*<sup>35</sup>. That cholesterol removal facilitates acrosome reaction is also substantiated in spermatozoa of many other species by using cholesterol-depleting agents like  $\beta$ -cyclodextrin<sup>36-38</sup>. In this context, mention should be made here of the bovine seminal plasma proteins (BSPs) which have been widely characterized in bovine species, although similar antigens have, however, been detected in several other species like stallion, boar, rat, mouse, hamster and human<sup>35</sup>. These proteins have been found to be deposited on the surface of spermatozoa and they also bind to the cholesterol effluxing agents like heparin and high density lipoproteins (HDL). It is, therefore, hypothesized that BSPs binding to spermatozoa increases the binding site of these cholesterol effluxing agents in the female tract, thus providing the trigger for capacitation. Cholesterol removal increases the sperm membrane fluidity<sup>39</sup>, and as a consequence alterations in the biophysical properties of the membrane would occur, which could obviously trigger the transmembrane signaling during capacitation. Besides, the increase in sperm membrane fluidity has also been correlated with lateral reorganization of the membrane proteins<sup>40,41</sup>,

and with increased permeability of the membrane to ions essential for capacitation<sup>42</sup>.

### First messengers

**Progesterone**—Since capacitation occurs in the female reproductive tract, attempts have been made to identify physiological inducers of capacitation in the tract. The most obvious candidates appear to be the female reproductive hormones such as progesterone; this hormone is present in high concentration in the cumulus matrix surrounding the oocyte and in the follicular fluid, and is capable of inducing acrosome reaction in spermatozoa of almost all species studied<sup>43-45</sup>. The effect of progesterone was not mediated through its cytoplasmic receptor, thus indicating that its ability to induce acrosome reaction is a non genomic effect<sup>46</sup>. The non genomic effect of progesterone could be due to its ability to perturb membranes, induce fluidity changes, induce membrane fusion<sup>47</sup> and also to induce uptake of calcium<sup>48</sup> by interacting with surface receptors on the spermatozoa. In human spermatozoa, two surface receptors have been identified with different affinities for progesterone<sup>49</sup>. Progesterone receptor mRNA has also been detected in human spermatozoa<sup>50</sup>.

**Bicarbonate ions**—Bicarbonate is a major component of the uterine and oviductal fluid<sup>51</sup>, and Lee and Storey<sup>52</sup> unequivocally established bicarbonate ion as a first messenger in the process of capacitation based on the observation that replacement of the bicarbonate buffer in *in vitro* fertilization medium with HEPES buffer effectively inhibited fertilization. It has been thus proved that bicarbonate is required for capacitation-associated processes like hyperactivation and tyrosine phosphorylation, and its effect on hyperactivation has recently been quantified<sup>53</sup>. Visconti *et al.* have demonstrated that the absence of bicarbonate ions could be overcome by cAMP analogues during capacitation in mouse<sup>54</sup> and hamster<sup>55</sup> suggesting that cAMP is the second messenger for bicarbonate ions in capacitation. The recent cloning and characterization of the atypical soluble adenylyl cyclase of spermatozoa<sup>56,57</sup> have conclusively proved that it is triggered by bicarbonate. Other effects of bicarbonate which support its involvement in capacitation are its ability to induce hyperpolarizing current during capacitation<sup>58</sup>, ability to increase the sensitivity of

voltage dependant calcium channels<sup>59</sup>, ability to remodel the membrane lipid architecture during capacitation which is probably mediated through a bicarbonate phospholipid 'scramblase'<sup>60</sup>, and its ability to increase the intracellular alkalinity which is a very essential step early in capacitation<sup>61</sup>. The effects of bicarbonate could be blocked by anion transporter blockers, DIDS or SITS<sup>55</sup>. A sodium dependant chloride/bicarbonate exchanger has been implicated in bicarbonate transport into the spermatozoa<sup>61</sup> causing alkalinization during capacitation, whereas the hyperpolarizing effect of bicarbonate might be involving a sodium bicarbonate co-transporter<sup>58</sup>. Recently, the migration of CRISP-1 to the equatorial segment during capacitation of rat spermatozoa has been found to be bicarbonate ion dependent<sup>62</sup>. The manifold importance of bicarbonate ion in sperm capacitation *in vivo* became evident in an interesting report wherein the investigators postulate that the low female fertility in cystic fibrosis patients could be due to hampered uterine secretion of bicarbonate ions through defective cystic fibrosis transmembrane receptor (CFTR) in the patients, thus causing insufficient sperm capacitation<sup>63</sup>.

**Others**—Heparin has been well characterized as a first messenger in bovine species<sup>64</sup> and is thought to cause cholesterol efflux from the spermatozoa plasma membrane by binding to the already membrane bound BSPs (mentioned before). An interesting class of agonists, the Fertilization Promoting Peptide (FPP) and adenosine, stimulates adenylyl cyclase activity in non-capacitated spermatozoa, but inhibits its activity in capacitated spermatozoa<sup>65</sup>. A dual function like this is probably accomplished by the first messengers affecting either through a stimulatory (A2) or an inhibitory (A1) receptor, depending on the status of capacitation<sup>66</sup>. Gamma amino butyric acid (GABA) has been recently emerging as a first messenger during sperm capacitation in ram<sup>67</sup>, bovine species<sup>68</sup> and in human<sup>69</sup>. Although not well characterized, neuraminidase<sup>70</sup>, piperine<sup>71</sup>, interleukin 6<sup>72</sup>, platelet activating factor<sup>73</sup>, atrial natriuretic peptide<sup>74</sup>, and epidermal growth factor<sup>75</sup> have also been shown to induce capacitation in various species.

### Second messengers

**Calcium ions**—Calcium is known to influence various functions of the spermatozoa such as motility,

capacitation and fertilizing ability. Elevation of calcium in the flagellum of spermatozoa drives hyperactivation in many species<sup>7</sup>, and this action of calcium could be at the level of the sperm flagella as revealed by experiments with demembrated rat<sup>76</sup> and bull<sup>77</sup> spermatozoa. Calcium is also indispensable for acrosome reaction. Acrosome itself is thought to be a calcium store which is proposed to release calcium during acrosome reaction by activating PKA and IP3 dependent calcium channels in the outer acrosomal membrane<sup>78</sup>. Intracellular calcium surge in spermatozoa occurs at two stages, first from the internal stores and later from the extracellular medium<sup>79</sup>. Disruption of some voltage or cyclic nucleotide gated calcium channels, previously identified in sperm, has failed to render the animal infertile<sup>80</sup>. But, in this context stands out the discovery of Catsper 1 to 4, which are sperm specific calcium channels localized to the sperm flagellum<sup>80-83</sup>; knock-out male mice of Catsper(s) have been found to be defective in hyperactivation and thus, infertile. The role of calcium in the capacitation dependent tyrosine phosphorylation event is quite controversial. Some researchers have shown that increase in calcium concentration in the medium causes an increase in tyrosine phosphorylation in mouse<sup>54</sup> and human<sup>84</sup> spermatozoa, while contrasting reports (decrease in tyrosine phosphorylation with increase in calcium) also exist. The decrease in tyrosine phosphorylation by calcium could be due to its inhibitory effect on kinases<sup>85</sup> or by decreasing the availability of ATP by activating calcium dependent ATPases in the spermatozoa<sup>86</sup>. In hamster, calcium has been shown to be required for hyperactivation and acrosome reaction, but not for tyrosine phosphorylation during capacitation<sup>13</sup>.

*Cyclic adenosine monophosphate (cAMP)*—The intracellular level of cAMP, which has been first recognized as a probable player in capacitation by Toyoda and Chang<sup>87</sup> and Rosado *et al.*<sup>88</sup>, has now been shown to increase in spermatozoa during capacitation<sup>89</sup>. This increase could probably be due to both increased activity of adenylyl cyclase, and inhibition of the activity of cAMP phosphodiesterases<sup>90</sup>. The increase in cAMP has also been shown to be dependent on calcium/calmodulin system<sup>89,91</sup> as well as on bicarbonate ion<sup>54</sup>. The sperm adenylyl cyclase is different from the somatic form in that it exists in many isoforms and is present both in

the membrane fraction and in the soluble fraction<sup>92</sup>; further, it is not coupled to any G protein<sup>93,94</sup> and is also insensitive to hormonal regulation or fluoride<sup>95</sup>. Buck *et al.* have purified a truncated form of a soluble adenylyl cyclase from rat testis and they have suggested that the lower molecular mass of the enzyme is due to post-translational processing mechanisms<sup>56</sup>. This soluble enzyme was highly responsive to bicarbonate stimulation (and not to forskolin), which might explain the increase in cAMP levels upon bicarbonate stimulation, and is also substantially homologous to the cyanobacterial enzyme. However, doubts have been expressed over the existence of the truncated soluble form calling it an experimental artifact<sup>90</sup>. Nevertheless, the two well established molecular effects of cAMP during capacitation are—(a) alteration of membrane phospholipid architecture (probably transduces the signal from the bicarbonate ions<sup>96</sup>); and (b) protein tyrosine phosphorylation<sup>90</sup>.

#### Down stream events

*Intracellular alkalinization and hyperpolarization*—Studies have earlier shown that increased cytosolic pH promotes metabolic activity and swimming activity of ejaculated spermatozoa and the increased intracellular alkalinization resulted from increased extracellular potassium, presumably a consequence of membrane depolarization<sup>97,98</sup>. In mouse sperm, the alkalinization has been shown to involve a sodium dependent chloride bicarbonate exchanger<sup>61</sup>, while in bovine spermatozoa, glucose has been shown to cause a delay in capacitation by delaying the intracellular alkalinization<sup>99</sup>. Alkalinization of the acrosome has also been correlated to the acrosome reaction in mouse and hamster spermatozoa<sup>28</sup>. However, the exact stimuli that bring about the change in pH are still unknown.

Hyperpolarization of the sperm plasma membrane has also been shown to accompany capacitation in mouse spermatozoa (through involvement of a bicarbonate sodium co-transporter)<sup>58</sup> and in bovine spermatozoa (by alteration of potassium permeability)<sup>100</sup>. The capacitation dependent hyperpolarization has been suggested to be an indirect trigger for protein tyrosine phosphorylation<sup>58</sup> and has also been shown to recruit calcium channels from an inactivated to an activable state which could be triggered later by the right stimuli like zona

pellucida<sup>101,102</sup>. Progesterone stimulation was also shown to cause hyperpolarization in a certain population of human spermatozoa<sup>103</sup>.

*Membrane reorganization*—Capacitation, triggered by a cholesterol efflux, is thought to render the male gamete “more fusible” to the female gamete by reorganization of the membrane components<sup>104</sup>. It has been recently demonstrated that the plasma membrane lipid architecture is controlled via a bicarbonate-cAMP-PKA dependent mechanism during capacitation in boar spermatozoa<sup>96,105,106</sup>. A caspase independent and bicarbonate dependent externalization of phosphatidylserine and phosphatidylethanolamine in human sperm membrane<sup>107</sup> has also been demonstrated. Recently, a novel aminophospholipid transporter in the acrosomal region of spermatozoa has been discovered and shown to be critical for normal phospholipid distribution in the membrane bilayer, and for normal binding, penetration, and signaling by the zona pellucida<sup>108</sup>. In addition to lipids, some membrane proteins also undergo reorganization during capacitation. PH-20 can be cited as a very good example; the protein migrates to the inner acrosomal membrane from the posterior head domain after acrosome reaction<sup>109</sup>.

*Reactive oxygen species (ROS)*—Over the years, the ROS, superoxide anion and hydrogen peroxide have been implicated in sperm capacitation<sup>110</sup>. These ROS have been shown to induce hyperactivation<sup>111</sup>, acrosome reaction<sup>112-114</sup>, and protein tyrosine phosphorylation which lies at the heart of the process of capacitation of spermatozoa<sup>115,116</sup>. The action of ROS is probably mediated by its ability to induce cAMP generation<sup>117</sup> and by altering the sulphhydryl levels in sperm proteins<sup>118</sup>. The ROS generating machinery in spermatozoa is uncharacterized till now, but the NADPH oxidase system in spermatozoa has been hinted upon to be responsible for generating ROS in rat<sup>119</sup> and human spermatozoa<sup>120</sup>, although contradictory evidence to it also exists<sup>121</sup>.

*Nitric oxide (NO)*—Although high concentrations of the free radical, NO, have been reported to decrease viability of spermatozoa by inhibiting respiration<sup>122,123</sup>, lower concentrations of NO, however, seem to facilitate capacitation dependent events such as motility enhancement<sup>124,125</sup>, stimulation of acrosome reaction in mouse spermatozoa<sup>126</sup> and enhancement of the zona pellucida binding ability in

human spermatozoa<sup>127</sup>. NO inside a cell is mainly generated by the action of nitric oxide synthase (NOS) and inhibitors of the enzyme have been used to show that NO regulates capacitation dependent protein tyrosine phosphorylation in spermatozoa, and thus probably affects the capacitation dependent events<sup>128,129</sup>.

*Protein tyrosine phosphorylation*—The backbone of phenotypic changes in spermatozoa during capacitation is thought to be the concomitant tyrosine phosphorylation in an array of sperm proteins. First demonstrated by Visconti and his co-workers<sup>54</sup>, the capacitation associated protein tyrosine phosphorylation in mouse spermatozoa has now been examined and verified in spermatozoa from many other species<sup>13,22,37,54,130-132</sup>; however, the array of proteins that get phosphorylated varies from species to species. The protein tyrosine phosphorylation event has been found to be dependent on BSA, and other cholesterol acceptors<sup>13,54</sup> and can be abolished in presence of certain tyrosine kinase inhibitors<sup>133</sup>. The follicular fluid of the female tract also induces protein tyrosine phosphorylation<sup>134</sup>, and, on the other hand, an epididymal protein CRISP-1 has been identified which when added to spermatozoa can inhibit capacitation dependent tyrosine phosphorylation<sup>135</sup>. These findings prove the *in vivo* relevance of the protein tyrosine phosphorylation event. The role of bicarbonate, calcium, cAMP and reactive oxygen species on capacitation dependent protein tyrosine phosphorylation have been dealt with in the respective sections above. Metabolic components of *in vitro* capacitation medium, like glucose, have also been shown to influence tyrosine phosphorylation in mouse, and glycolysis has been shown to be the source of ATP for protein phosphorylation<sup>136</sup>. Almost in all species studied, immunolocalization studies have indicated that the capacitation dependent tyrosine phosphorylation goes up in the flagella of the spermatozoa<sup>116,132,137-139</sup>. Although the essentiality of a balance between protein tyrosine kinase and phosphatase activities has been demonstrated in causing a successful acrosome reaction<sup>140</sup>, the tyrosine phosphorylated proteins involved in the process are yet to be identified.

Sperm protein tyrosine phosphorylation is triggered by high levels of cAMP, which regulate protein kinase A (PKA) and role of PKA has also been

investigated and proved in various species<sup>141</sup>. Involvement of PKA is indicated since inhibitors of PKA activity are able to inhibit tyrosine phosphorylation as well as capacitation<sup>55,142</sup>. As PKA is a serine kinase, one or more intermediate tyrosine kinases or phosphatases have been proposed to be involved in the tyrosine phosphorylation event, whose activities could be regulated by PKA. Involvement of calcium in capacitation necessitated the investigation of calcium stimulated kinase, protein kinase C (PKC), in capacitation or in related events. By now, involvement of PKC in acrosome reaction is reasonably well worked out. Immunolabelling studies have revealed different locations of the kinase in different regions of acrosome in different species<sup>78</sup>. Involvement of PKC has been established by using phorbol ester (PMA) as a potent stimulator (and other weaker stimulators) to induce acrosome reaction in a PKC dependent manner which could be brought down by PKC inhibitors. Moreover, PKC has also been implicated in the acrosome reaction stimulated by physiological agents like progesterone and zona pellucida<sup>28</sup>. Stimulation of PKC is proposed to be driven by diacyl glycerol produced by the action of phospholipases on the membrane phospholipids<sup>78</sup>. Although protein tyrosine phosphorylation has been accepted as a hallmark of capacitation, direct involvement of any particular tyrosine kinase has not been shown in sperm capacitation. Epidermal Growth Factor Receptor, a receptor tyrosine kinase, has been detected in the head of the bovine spermatozoa and EGF induced acrosome reaction in the same species has been found to be completely inhibited by tyrosine kinase inhibitors<sup>143</sup>. In hamster spermatozoa, components of the *Ras* pathway have been detected although their functional significance is still a matter of conjecture<sup>144</sup>. Non-receptor tyrosine kinase c-yes has been shown to be highly expressed in the spermatid acrosome among many mammalian tissues studied<sup>145</sup> and its activity appears to be positively and negatively regulated by cAMP, and calcium, respectively, during human sperm capacitation<sup>146</sup>. Partial purification of a tyrosine kinase has also been done from hamster spermatozoa which might be responsible for capacitation dependent signaling event<sup>8</sup>. To increase the net tyrosine phosphorylation in a cell, the normal balance between the kinases and the

phosphatases has to be tilted; thus, studies on the phosphatases are as important as that on kinases. An isoform of PPI (PPI gamma) or the inhibitor of the PKA regulatory subunit has been identified in spermatozoa<sup>147</sup> and has been found to be associated with AKAP220 in the spermatozoa. However, another abundant isoform of the same inhibitor, PPI beta, when knocked out, does not show any detectable infertility in both male and female mice<sup>148</sup>. Calcium/calmodulin dependent phosphatase, calcineurin (PP2B), has been detected in dog spermatozoa<sup>149</sup> and also appears to participate in dephosphorylation of tyrosine phosphorylated substrates in human spermatozoa<sup>150</sup>. This area of research demands more focus and would eventually help in a better understanding of the regulation of the phenomenon of capacitation in spermatozoa.

Each tyrosine-phosphorylated protein in sperm is a tool to understand the cause and effect relationship between tyrosine phosphorylation and capacitation. However, till now, only a few of these proteins have been identified, and any functional significance of tyrosine phosphorylation is attached even to a fewer of such proteins. The most characterized protein in this aspect is the Kinase A anchoring protein 82 (AKAP82/AKAP4) which has been found to be the major structural protein in the fibrous sheath of spermatozoa<sup>151</sup>. This protein has been identified as a capacitation dependant phosphorylated protein in human<sup>150</sup>, mouse<sup>151</sup>, rat<sup>152</sup> and hamster<sup>137</sup> spermatozoa, although the type of phosphorylation (tyrosine/serine) varied depending on the species; in human and hamster this protein has been shown to be tyrosine phosphorylated and in mouse, it is serine phosphorylated. The state of phosphorylation of the AKAP could be a variant in the docking of the kinase (PKA), which would thus facilitate its localized action. However, this theory requires evidence. Recently, the tyrosine phosphorylation of another AKAP3 has been shown to result in the recruitment of protein kinase A to the sperm flagella causing an increase in motility<sup>153</sup>. It is worthwhile mentioning here that AKAP isoforms have also been localized in the acrosome of mammalian spermatozoa<sup>154,155</sup>.

A novel testis specific protein designated as calcium-binding tyrosine phosphorylation-regulated

protein (CABYR) whose calcium binding property has been shown to be dependent on tyrosine phosphorylation<sup>156</sup>, was found to be localized in the fibrous sheath of human spermatozoa. A chaperone, HSP90 $\alpha$ <sup>157</sup> and a mitochondrial sheath protein, phospholipid hydroperoxide glutathione peroxidase (PHGPx)<sup>158</sup> have also been identified as tyrosine phosphorylated proteins in capacitated mouse, and hamster spermatozoa, respectively. A global phosphoproteome analysis of human spermatozoa<sup>159</sup> led to the identification of a set of eighteen proteins, which get tyrosine phosphorylated during sperm capacitation, which includes ion channels, metabolic enzymes, structural proteins etc, and also HSP90 $\alpha$  and PHGPx. In this report, a valosin containing protein (VCP) has been looked into more detail among this set of proteins revealing relocation of the protein from the neck in the non-capacitated to the anterior head region in the capacitated human spermatozoa. In hamster spermatozoa, dihydrolipoamide dehydrogenase, a metabolic enzyme, has been detected as a capacitation dependent tyrosine phosphorylated protein and its involvement in hyperactivation and acrosome reaction has also been demonstrated<sup>160</sup>.

### Metabolism during capacitation

The metabolism of spermatozoa is unique compared to other cells because of two primary reasons; (a) presence of sperm specific isoforms of some metabolic enzymes<sup>161-163</sup>, and (b) the metabolism in sperm is compartmentalized with the mitochondrial Electron Transport Chain (ETC) in the midpiece and the glycolytic apparatus in the principal piece<sup>136</sup>. In mouse and man, glucose has been shown to be the primary carbon source and glycolysis to be the primary metabolic pathway for capacitation of spermatozoa<sup>10,164</sup>. On the other hand, in guinea pig spermatozoa, the role of glycolytic pathway is not clear as there are contradicting reports about the positive role of glucose<sup>165,166</sup>. In human spermatozoa, it is postulated that glucose could be linked to the increase in super oxide generation, which is an essential step in hyperactivation and protein phosphorylation during capacitation<sup>120</sup>. In mouse spermatozoa<sup>136</sup>, glycolysis, and not ETC has been indicated as a source of ATP for phosphorylation. The

oxygen consumption has also been shown to decline during capacitation<sup>167</sup>. In a very recent report in which the knock-out mice of the sperm specific glyceraldehyde 3-phosphate dehydrogenase (GAPDH-S) were found to be infertile, it was also shown that the oxygen consumption in the sperm of the knock-out animals was normal<sup>168</sup>. In hamster, it has been recently shown that all three carbon sources present in *in vitro* capacitation medium namely glucose, pyruvate and lactate are required during capacitation; the former is required in the early hours and the latter two at the later hours of capacitation<sup>169</sup>.

### Conclusion

Although a good number of molecules have been identified and implicated in capacitation, the available information is not enough to understand the phenomenon of capacitation completely. There are many un-addressed open questions which need to be answered for a better understanding of capacitation. Studies adopting many different approaches have added several other candidate proteins to the complex scenario of capacitation (Table 1). These proteins are, however, not properly characterized. Figure 1 gives the schematic representation of the molecular basis of capacitation. Since spermatozoa are highly arrested at the level of transcription and translation, the intracellular signaling of information during sperm capacitation takes place through post-translational modifications of proteins like tyrosine phosphorylation. Therefore, process of capacitation dependent tyrosine phosphoproteome of spermatozoa may unravel through signal transduction. Thus, identification of different tyrosine phosphorylated proteins is essential. On the other hand, our knowledge about the involvement of the metabolic components, especially the enzymes of the post pyruvate-lactate metabolism, in capacitation is very fragmentary and scanty. However, a link between the metabolic components and the signal transduction components in spermatozoa has begun to reveal itself through glucose dependent tyrosine phosphorylation and metabolic enzymes getting tyrosine phosphorylated during capacitation. Another untouched area is the identification of the PKA dependent tyrosine phosphatases and tyrosine kinases which are directly involved in protein tyrosine phosphorylation during capacitation. Thus,

Table 1—List of proteins* implicated in capacitation			
Name of the candidate protein	Involvement (species)	Experimental basis	References
Sperm c-myc proto oncogene product	Hyperactivation and penetration of zona-free hamster eggs (human); <i>in-vitro</i> fertilization (mouse); <i>in-vivo</i> fertilization (rabbit)	Effects observed by using a monoclonal antibody	170
Soluble acrosome inducing factor in egg-cumulus complex	ZP induced acrosome reaction and spermatozoa penetration (hamster)	Inducer used was soluble extracts of ovulated egg-cumulus	171
A guanine-nucleotide binding regulatory protein	ZP induced acrosome reaction (human)	Effects observed by using inhibitor (pertussis toxin)	172
Phospholipase A2	Acrosome reaction and fertilizing ability (hamster)	Effects observed using antibody	173
Lipid transfer protein1	Capacitation (human)	Purified protein augments capacitation	174
Glycine receptor/Chloride Channel	ZP induced acrosome reaction (human, porcine and mouse)	#Effects observed using channel blocker (strychnine) and agonists (glycine) #Transgenic studies	175,176
Calmodulin	Capacitation and ZP induced acrosome reaction (mouse)	CaM antagonists (calmodulin binding domain, calmidazolium, compound 48/80, ophiobolin A, W5, W7, and W13)	177
FSP95	Capacitation (human)	Undergoes tyrosine phosphorylation	178
Rab3a	ZP induced acrosome reaction (mouse)	Localization studies	179
LDH C-4	Capacitation (bovine)	Metabolic studies	181
gC1q-R/p33	Capacitation (human)	Expression in the plasma membrane increases with capacitation	182
Caveolin-1	Capacitation (guinea pig)	Localization in the rafts	183
<i>GAPDH-S</i>	Motility and fertility (mice)	Knock out studies	168
Plasma membrane associated hyaluronidase	Acrosome reaction (stallion)	Localization studies	184
Sp17 (calmodulin binding protein)	Acrosome reaction (rabbit)	Processing during acrosome reaction	185
T-type calcium channels	Progesterone induced capacitation (mouse)	Results observed by using channel blocker (NiCl <sub>2</sub> )	186
PI3K	Negatively regulates hyperactivation (human)	Inhibitor, LY294002, enhances motility	187
VLA-6 integrin	Cumulus (laminin) induced acrosome reaction (porcine)	Localization studies	188
Calcitonin	Capacitation and <i>in-vitro</i> fertilization (mouse)	Peptide enhances capacitation (CTC)	189
Angiotensin II	Capacitation and <i>in-vitro</i> fertilization (mouse)	Peptide enhances capacitation (CTC)	189
SVS VII phospholipid binding protein	Motility (mouse)	Binding enhances motility	190
Vitronectin	Acrosome reaction (human)	Localization studies associated with acrosome reaction	191
Glycosyl transferases	Modification of spermatozoa surface glycoproteins (hamster)	Enhanced activity during estrus	192

\*Proteins not mentioned in the text are only mentioned.

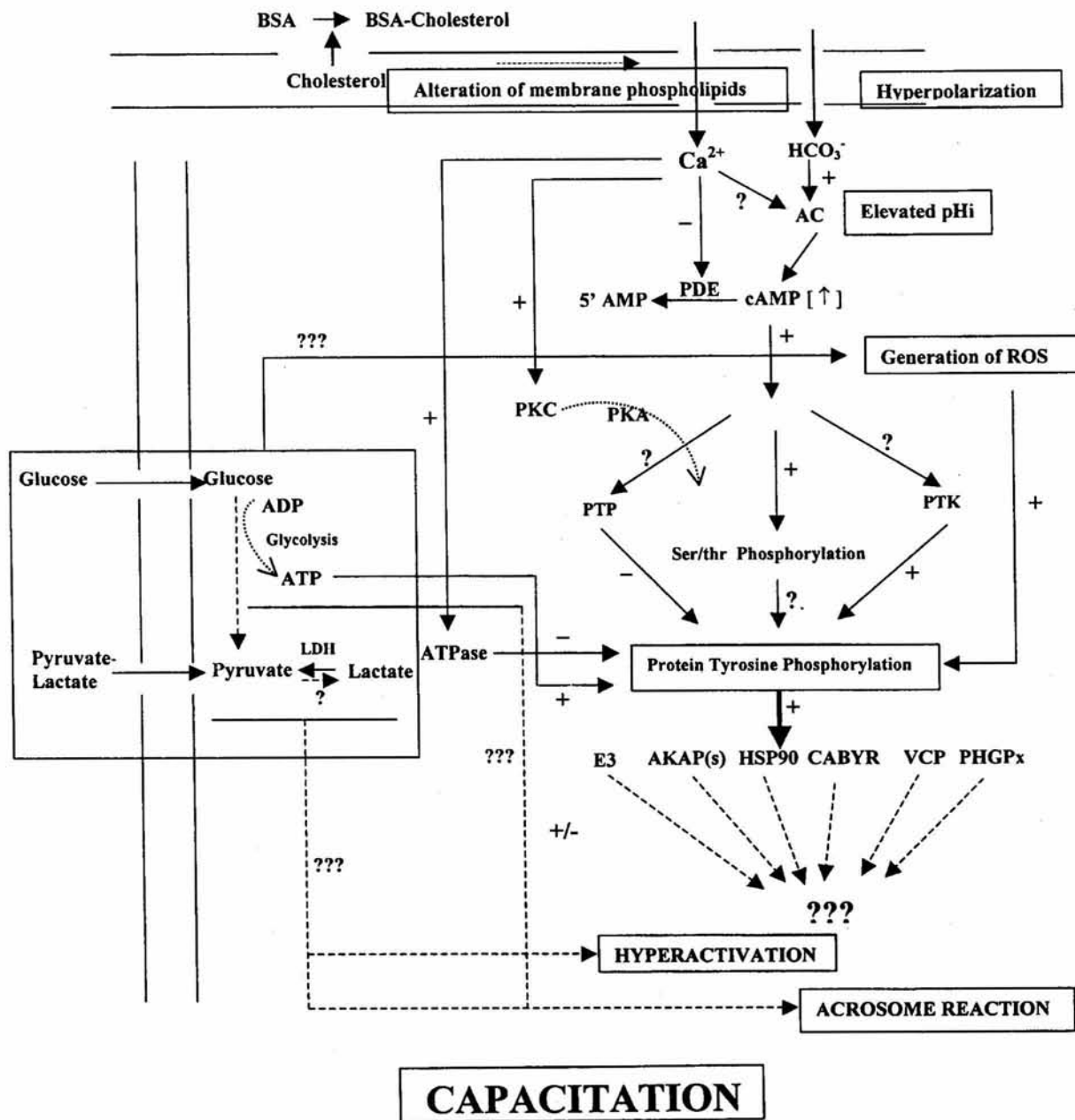


Fig. 1—Schematic representation of the signaling components with protein tyrosine phosphorylation at the backbone. The cellular changes happening during capacitation are boxed. Hallmarks of capacitation are also boxed and are in capitals. Cholesterol effluxing agents like BSA, turn on capacitation and thereafter bicarbonate ( $\text{HCO}_3^-$ ) induces adenylate cyclase (AC) to synthesize cyclic AMP (cAMP). Protein kinase A (PKA) is then triggered by cAMP which drives protein tyrosine phosphorylation in an array of sperm proteins. The tyrosine phosphorylated protein tyrosines which have been identified are listed. The metabolic components of capacitation and their links with the signaling components of capacitation are also mentioned. Modified from Jha and Shivaji<sup>193</sup>.

understanding capacitation would help not only to understand the intricacies of sperm function, but could also open up avenues for contraception oriented research.

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