Localization patterns of the ganglioside G_{M1} identify sperm capable of undergoing capacitation and acrosome exocytosis

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Abstract

Introduction: Sperm must first capacitate and then undergo acrosome exocytosis (AE) to fertilize. Localization patterns of G_{M1} change in response to capacitation stimuli and are quantified using the Cap-Score™; this result tracks strongly with human clinical fertility. This association supported the notion that individual human sperm showing "capacitated" G_{M1} patterns represented capacitated sperm. Here, we examined directly if "capacitated" G_{M1} patterns represent cells that are capacitated and can undergo AE.
Methods: 10 semen samples were collected from fertile men (pregnant partner or recent father). Samples were liquefied, washed, and aliquots incubated under non-capacitating (NC) and capacitating (CAP) conditions. For 7 of these samples, a CAP+calcium ionophore A23187 treatment was used to induce AE. In a separate experiment, NC (n=5) and NC+calcium ionophore A23187 (n=5) treatments were also prepared. For all samples, cells were attached to slides and dual labeled with PNA, a lectin that binds specific carbohydrate residues associated with the acrosome, and Cholera Toxin B to determine G_{M1} localization and capacitation status.
Results: Whether under NC or CAP conditions, most cells with non-capacitated G_{M1} localization patterns (G_{M1}NC) did not label with PNA (NC: 58.2±6.6%; CAP: 45.9±6.0%). Interestingly, whether under NC or CAP conditions, the majority of cells with the Capacitated G_{M1} localization pattern (G_{M1}CAP) labeled with PNA over the acrosome (NC: 53.6±7.1%; CAP 63.1±4.4%). These data are consistent with knowledge of plasma membrane dynamics during capacitation, in which point-fusions occur with the underlying outer acrosomal membrane, exposing acrosomal matrix contents. Samples exposed to CAP+Ionophore showed a decrease in Cap-Score when compared to CAP samples (CAP: 28.0±2.6%, CAP+Ionophore: 19.4±1.4%, p=0.02). No decrease in Cap-Score was observed in the NC+Ionophore treatment (NC: 15.9±2.9%, NC+Ionophore: 15.7±0.9%, p= 0.96). These findings are consistent with sperm undergoing acrosome exocytosis originating from the sub-population that would otherwise have had a G_{M1}CAP pattern and corroborates prior data using mouse sperm.
Conclusion: On a single cell level, sperm having the "G_{M1}CAP" pattern are those that responded to capacitation stimuli and those that underwent AE. These data substantiate earlier clinical studies on populations of sperm, linking the Cap-Score with sperm function and male fertility.

Introduction

Sperm must first capacitate and then acrosome react to be fertilization competent. Previous studies suggest that localization of the ganglioside G_{M1} (Cap-Score™-proportion of cell within a ejaculate that are deemed capacitation competent) can identify sperm capable of capacitating and strongly correlates with male fertility. Here we validated that G_{M1} localization patterns reflect capacitation in human sperm. First, dual label with PNA and CTB were performed to determine whether sperm having the patterns believed to relate to a capacitated state showed accepted signs of capacitation. Then we incubated sperm with capacitation stimuli and calcium ionophore A23187. If the Cap-Score reflects the sperm that were capacitated, it is hypothesized that the Cap-Score should decrease upon treatment with ionophore. The decrease in Cap-Score would result because the process of exocytosis would disrupt the plasma membrane overlying the acrosome, preventing labeling or leading to atypical patterns.

Results

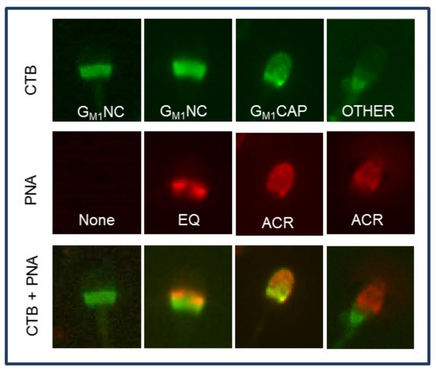

Figure 1. ***PNA and G_{M1} localization patterns in human sperm.*** Images of fluorescently labeled sperm showing non-capacitated G_{M1} localization patterns predominantly found in sperm incubated under non-capacitating conditions (G_{M1}NC); a pattern of G_{M1} localization that increased in response to incubation with stimuli for capacitation (G_{M1}CAP); and a pattern of G_{M1} localization that appeared infrequently (OTHER). Three patterns of PNA labeling were commonly seen in cells that also labeled with CTB: no labeling of PNA (None), labeling over the equatorial region (EQ); and labeling over the acrosome (ACR).

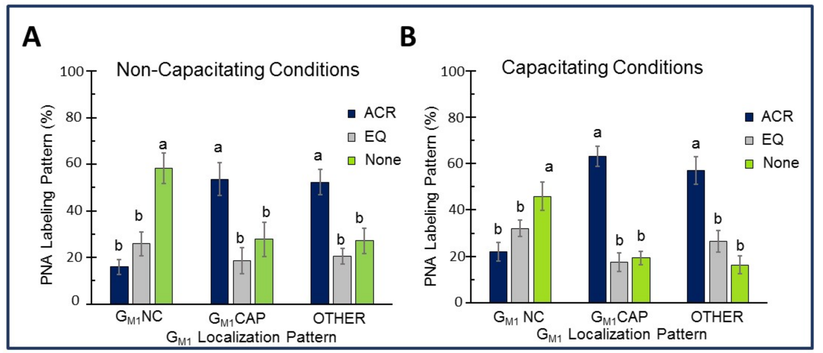

Figure 2. ***Relationship between G_{M1} localization patterns, capacitation status and exposure of acrosomal carbohydrates as detected with PNA.*** G_{M1} localization patterns were compared against PNA labeling in sperm incubated under non-capacitating (NC, n=10) and Capacitating (CAP, n=10) conditions, shown in **panels** (**A**) and (**B**) respectively. The x-axes show the patterns of G_{M1} localization. The blue, grey and green bars represent the proportion of cells with that CTB labeling pattern having ACR, EQ, and None PNA label respectively. Means within a given G_{M1} localization pattern were compared. Means with different superscripts were found to be different using Fisher's LSD ($p<0.05$). Under NC and CAP conditions, most of the cells with the G_{M1}NC pattern did not label with PNA (58.2±6.6% NC conditions; 45.9±6.0% CAP conditions). The percentage of sperm having the G_{M1} "Capacitated" pattern (G_{M1}CAP) increased from 16.3±2.5% (n=10) under NC conditions, to 28.0±2.6% (n=10) after 3 hours under CAP conditions ($p<0.01$). Under CAP conditions, the majority (63.1±4.4%) of cells with the G_{M1}CAP pattern labeled with PNA over the acrosome. Only 19.3±2.8% had no label, and 17.6±4.0% had equatorial label.

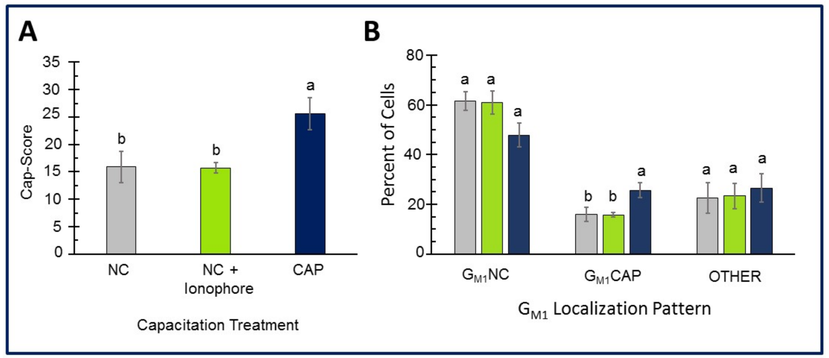

Figure 3. ***Effect of calcium ionophore A23187 on Cap-Score under NC conditions.*** Sperm were incubated in basal, non-capacitating medium (NC; n=5), or with non-capacitating medium and then incubated with A23187 (NC+Ionophore; n=5), or with stimuli for capacitation (CAP; n=4;). **(A)** Bar graph shows the Cap-Score (percent of cell showing G_{M1} capacitated patterns) obtained for each treatment. No difference in Cap-Score was observed between the NC and NC+Ionophore treatments, substantiating a lack of exocytosis in non-stimulated sperm ($p=0.96$). **(B)** Reflects the changes in G_{M1} localization patterns that led to the change in Cap-Score. The x-axis shows the G_{M1} localization pattern and the y-axis shows the percent of cells having that pattern for each of the following capacitation treatments NC (gray), NC+Ionophore (green), and CAP (blue), the proportion of cells within the G_{M1} localization pattern were compared. Those means with different superscripts were found to be different using Fisher's LSD ($p<0.05$).

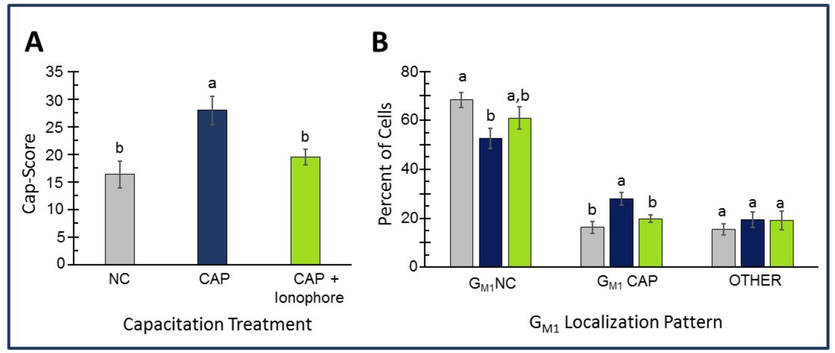

Figure 4. ***Effect of calcium ionophore A23187 on Cap-Score under CAP conditions.*** Sperm were incubated in basal, non-capacitating medium (NC; n=10), or with stimuli for capacitation (CAP; n=10), or with stimuli for capacitation and then with calcium ionophore (A23187) (CAP+Ionophore; n=7). **(A)** Bar graph shows the Cap-Score (percent of cell showing G_{M1} capacitated patterns) obtained for each treatment. An increase in Cap-Score was observed from the NC to CAP treatment. In the CAP + Ionophore treatment there was a reduction in Cap-Score, showing that the sperm having undergone exocytosis came from the subpopulation of cells having the G_{M1}CAP pattern. **(B)** The x-axis shows the G_{M1} localization pattern and the y-axis shows the percent of cells having that pattern for each of the following capacitation treatments NC (grey), CAP (blue), and CAP+Ionophore (green). The proportion of cells within the G_{M1} localization pattern were compared. Those means with different superscripts were found to be different using Fisher's LSD ($p<0.05$).

Conclusions

- The majority of sperm having the G_{M1}NC pattern did not label with PNA. In contrast, sperm having the G_{M1}CAP pattern predominantly showed PNA labeling over the acrosome. This is consistent with point-fusions occurring between the plasma membrane and underlying outer acrosomal membrane in sperm having the G_{M1}CAP pattern and substantiates their capacitation status.
- Cap-Score decreased in sperm incubated under CAP+Ionophore conditions. This supports the notion that cells undergoing acrosome exocytosis originated from the sub-population that would otherwise have had a G_{M1}CAP pattern.
- The present study supports earlier clinical studies linking the Cap-Score with sperm function and male fertility.

Applications

Identification of defects in sperm function can potentially allow clinicians to:

- Personalize reproductive therapies;
- Evaluate return to fertility following varicocele correction, vasectomy reversal or other procedures likely to alter sperm production;
- Assess effects of supplements or handling techniques that might affect sperm quality.

Funding