Influence of human sperm motility and cryopreservation on the oocyte activating factor phospholipase C zeta.

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OBJECTIVE:

Phospholipase C zeta (PLCz) is considered to be the sperm-borne factor responsible for successful mammalian oocyte activation. Absence, abnormal expression or defective functional forms of PLCz have been linked to oocyte activation deficiency and failed fertilization. As such, PLCz could be used as a biomarker of human male infertility.

DESIGN:

This study was implemented to determine the proportion and specific pattern of PLCz localization in sperm and to examine the effect of motile sperm selection (experiment A) and sperm cryopreservation (experiment B).

MATERIALS & METHODS:

Fresh semen samples of proven oocyte activation capacity were obtained from 17 patients with informed written consent. For experiment A, processed sperm from 14 patients was either used directly for PLCz immunofluorescence or after selecting exclusively motile sperm by a swim-out procedure. For experiment B, selected motile sperm from 3 patients was stained either fresh or after freezing-thawing of the native sample. A new affinity purified polyclonal antibody was used which specifically recognizes the full-length PLCz. Only acrosome-intact sperm were analyzed by laser scanning confocal microscopy. Group comparisons were performed with the Wilcoxon rank test and a P-value of 0.05 was considered statistically significant.

RESULTS:

The localization pattern of PLCz varied greatly among different patients. Overall, full length PLCz appeared to be localized predominantly in the acrosomal region alone or the acrosomal and post-acrosomal region of human sperm. A significantly higher percentage of sperm was devoid of PLCz when no selection of motile sperm was performed ($4\pm 1\%$ versus $20\pm 6\%$) Cryopreservation didn't seem to affect PLCz positive proportion or its localization pattern.

Localization pattern	Selected motile sperm (mean% $\pm$ SEM)	Unselected sperm (mean% $\pm$ SEM)
Acrosomal	41 ± 4	45 ± 3
Acrosomal & post-acrosomal	30 ± 4	29 ± 4
Acrosomal & equatorial	7 ± 1^a	4 ± 1^b
Acrosomal & equatorial & post-acrosomal	9 ± 2	5 ± 1
Equatorial	2 ± 1	3 ± 1
Equatorial & post-acrosomal	4 ± 1	3 ± 1
Post-acrosomal	8 ± 2	11 ± 2

^a vs ^b $P < 0.05$

CONCLUSIONS:

Collectively, our data indicate that sperm motility correlates with the presence of PLCz in human sperm. Full-length PLCz exhibited a distinct pattern of localization which is preserved after sperm freezing and thawing. Future studies should address the impact of PLCz proportion and localization pattern on subsequent ART outcome.

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