

midase activity is a critical determinant of GC survival, were supported by studies with the ceramidase inhibitors, Both OE and D-MAPP significantly potentiated GC apoptosis induced by either serum starvation or by 50 mM C8-ceramide treatment. By comparison, L-MAPP (an inactive stereoisomer of D-MAPP) had no effect on cell death induced by either serum starvation or C8-ceramide. Acid ceramidase was expressed abundantly in granulosa cells and ovaries and its expression was significantly increased by gonadotropin in granulosa cells. OE significantly increased ceramide production ( $p < 0.05$ ), however, D-MAPP did not increase ceramide production from granulosa cells after 4 hours treatment.

**Conclusions:** 1) C8-ceramide induced apoptosis in all cells at 50 mM concentration while the same concentration of C8-ceramide caused only 28.6% of cell death. 2) Acid ceramidase was expressed abundantly in granulosa cells and ovaries and its expression was increased by PMSG and, decreased in corpora lutea. 3) Ceramidase inhibition significantly potentiated GC apoptosis induced by C8-ceramide or serum starvation. 4) Ceramide metabolism is a critical determinant of granulosa cell fate.

## O-19 Expression of Sperm-specific Cation Channel CatSper in Human Spermatozoa

KW Cheon, HK Byun<sup>1</sup>, JY Hong<sup>1</sup>, HS Lee<sup>1</sup>, YS Park<sup>1</sup>, JT Seo<sup>2</sup>

*Laboratory of Reproductive Biology and Infertility<sup>1</sup> and Department of Urology<sup>2</sup>,  
Samsung Cheil Hospital & Women's Healthcare Center,  
Sungkyunkwan University School of Medicine*

**Background & Objectives:** Numerous studies have demonstrated that calcium ion controls sperm motility and capacitation. CatSper, a newly identified cation channel is reported to exist in flagellum of mouse spermatozoa. CatSper is a sperm-specific calcium channel and plays a key role in the sperm motility and the fertility in mouse. In this study, we aimed to elucidate the expression and intracellular localization of CatSper in human spermatozoa.

**Method:** The sperm samples were isolated from semens of 30 patients. After the CASA, the viability test and the morphological test, samples were divided into two groups (normozoospermia and asthenozoospermia). Using this sperm sample, we performed RT-PCR for mRNA expression and fluorescent immunocytochemistry for protein expression.

**Results:** In all of the sperm samples, we could found the mRNA expression of CatSper. We compared the expression of CatSper mRNA with sperm motility and progressiveness by semi-quantitative analysis. In comparison of CatSper mRNA expression and sperm motility (not distinguishing dead sperm from whole sperm population), the mean value of CatSper mRNA expression (mean $\pm$ SD) was  $1.5 \pm 0.6$  in normozoospermia (n=15) and  $1.4 \pm 0.6$  in asthenozoospermia (n=15). When we compared the expression of CatSper mRNA with motility/viability ratio for excluding non-motile dead sperm (group A;  $>0.5$ , group B;  $\leq 0.5$ ), group A (n=19) was  $1.5 \pm 0.6$  whereas group B (n=11) was  $1.2 \pm 0.6$ . The mean value of group A was 25% higher than that of group B but it was not significantly different. In comparison of CatSper

mRNA expression with progressiveness (group A'; >1.7, group B'; ≤1.7), group A' (n=20) was  $1.6 \pm 1.0$  whereas group B' (n=10) was  $1.3 \pm 0.6$ . The mean value of group A' was 23% higher than that of group B' but there also was no statistical significance. We found that CatSper protein was dominantly expressed in the flagellum of human sperm. Using double fluorescent immunostaining with anti-CatSper and anti- $\beta$ -tubulin antibody, we concluded that CatSper protein was localized in connecting piece, mid-piece and principal piece of flagellum except end piece.

**Conclusions:** To our best knowledge, this is the first report to show the expression of CatSper mRNA and protein in human spermatozoa. We failed to show the significant difference of CatSper mRNA expression between normozoospermia and asthenozoospermia. But we found the tendency of increasing CatSper mRNA expression according to increase of motility/viability ratio and progressiveness in ejaculated sperm. Moreover, we found that CatSper protein was localized in the flagellum of human sperm. So, further studies will be focused on the function of CatSper and the mutation of CatSper gene, resulting in the functional loss or impairment of CatSper as a cation channel in human spermatozoa.