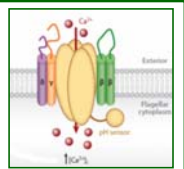


CATSPER CALCIUM CHANNELS IN HUMAN SPERMATOZOA AND THEIR ROLE IN RESPONSIVENESS TO PROGESTERONE (P).

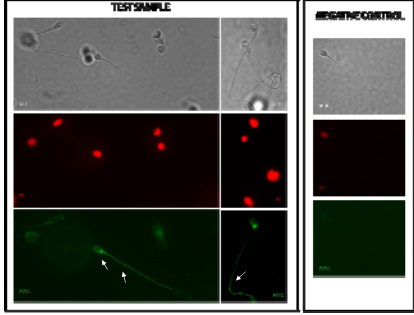
Tamburrino L, Marchiani S, Cambi M, Muratori M, Forti G, Baldi E.
Dept. of Biomedical Experimental and Clinical Sciences, University of Florence, Florence, Italy.

CATSPER is a family of sperm-specific calcium channels activated by P in human spermatozoa, and indicated as putative P sperm receptors (Strunker et al, 2011; Lishko et al, 2011). KO mice for CATSPER channels are infertile due to severe defects in sperm motility. The aim of this study is to investigate the occurrence of CATSPER-1 in human sperm and whether these channel is involved in human sperm motility and P-stimulated acrosome reaction (AR).



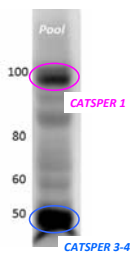
OCCURRENCE AND LOCALIZATION OF CATSPER IN HUMAN SPERM

Fluorescence Microscopy

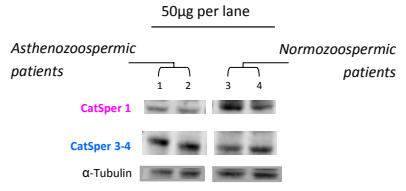


By immunofluorescence we observed that channels are mainly located in the principal piece of the tail

Western Blot



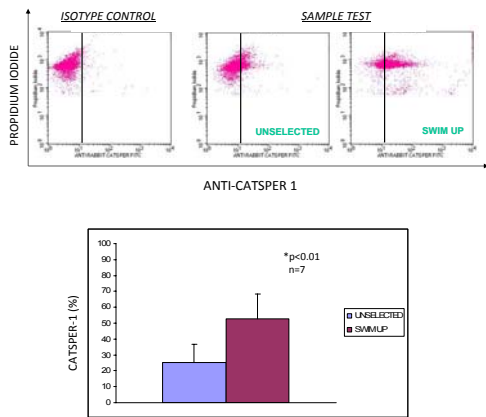
Western blot analysis demonstrated the presence of 2 major bands corresponding to CATSPER 1 and CATSPER 3-4



Asthenozoospermic patients show higher expression of CATSPER 3-4 compared to CATSPER 1 on the contrary of normozoospermic patients

ROLE OF CATSPER CHANNELS IN HUMAN SPERM MOTILITY

Flow Cytometry



By flow cytometry higher levels of CATSPER were found in swim up selected spermatozoa respect to unselected (52.7 ± 15.8 vs 25.4 ± 11.1 , $n=7$, $p=0.003$)

C.A.S.A.

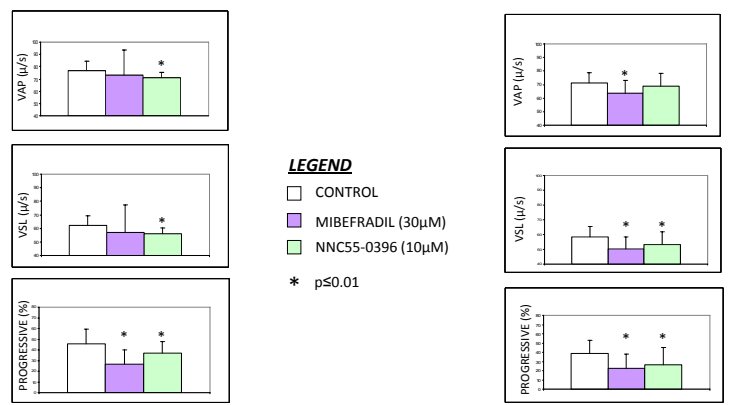
(COMPUTER ASSISTED SEMEN ANALYSER)



To investigate the role of CATSPER channels in human sperm motility, we evaluated the effects of the specific inhibitor NNC55-0396 (10 μ M) and the non specific inhibitor mibefradil (30 μ M) on swim up selected spermatozoa motility parameters, by using C.A.S.A.

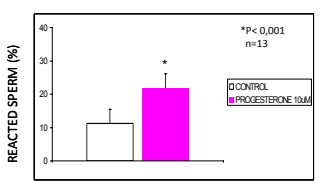
INHIBITORS ADDED TO SELECTED SPERM FOR 15 MIN AFTER CAPACITATION (n=10)

INHIBITORS ADDED DIRECTLY TO SPERM SWIM UP SELECTION MEDIUM (n=9)



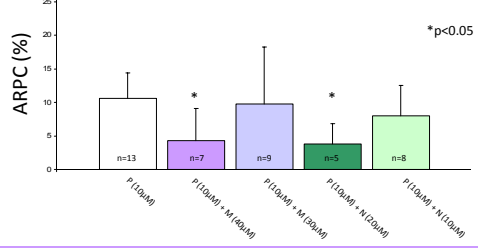
Both compounds significantly inhibited several motility-related parameters.

ROLE OF CATSPER CHANNELS IN P-STIMULATED AR



We evaluated the effect of mibefradil (M) and NNC55-0396 (N) on P (10 μ M)-stimulated AR in swim up selected human sperm.

Acrosome Reaction following Progesterone Challenge



Mibefradil at 30 μ M was ineffective, whereas a 50% inhibition was observed at 40 μ M. NNC55-0396 compound, tested at 20 μ M concentration, inhibited P-induced AR of 70%.

CONCLUSIONS

These results indicate that CATSPER calcium channels are involved in human sperm motility and P-induced AR. In light of a recent study (Jin et al, 2011) demonstrating that physiological AR occurs during transit in the cumulus matrix of the oocyte (where P is present at μ M concentrations) before sperm attachment to the zona pellucida, our data suggest that CATSPER may be considered a possible molecular target for the development of novel therapeutic strategies for male infertility as well as for male-directed contraception.

REFERENCES

- Strunker T, Goodwin N, Brenker C, Kashikar ND, Weyand J, Seifert R, Kaupp UB. The CatSper channel mediates progesterone-induced Ca²⁺ influx in human sperm. Nature. 2011 ;47:382-6.
- Lishko PV, Botchkina IL, Kirichok Y. Progesterone activates the principal Ca²⁺ channel of human sperm. Nature. 2011 ;471:387-391
- Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, Chiba K, Hirohashi N. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc Natl Acad Sci U S A. 2011 ;108:4892-6.