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Investigation of Ca^{2+} Channel Types in Human Spermatozoa

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AIM

Subfertility is a growing socioeconomic problem. The procedure of fertilization requires, among others, the fusion of a mature male with a female gamete. Non physiological male gametes comprise a major causes of subfertility. Several studies have shown that Ca^{2+} channels play a significant role in the motility of spermatozoa and the acrosome reaction. The aim of the present study is to determine the type of Ca^{2+} channels in human spermatozoa and more specific to investigate if the Ca^{2+} channels involved are potential- and/or receptor-dependent.

METHODS

Seminal specimens were collected from normozoospermic patients (n=15) after 42-36 h of sexual abstinence and allowed to liquefy for 15-30 min at room temperature. Each specimen was evaluated for count, percentage of motility, percentage of morphologically normal spermatozoa, according to standard methods recommended by the World Health Organization (WHO) (1), with phase contrast microscopy. All samples used in this experiment had at least 50% motile spermatozoa, 59% morphologically normal spermatozoa and spermatozoa concentrations of 50X 10^6/ml or greater. Each sample was processed for swim-up washing procedure. The swim-up technique (1) was used twice. The retrieved sperm pellet (0.3 ml) of each sample was resuspended in 3.7 ml of Universal IVF medium (Medicult). The final sperm samples showed forward motility percentages over 40%, normal sperm morphology more than 90%. Sperm concentrations were more than $5x10^6$/ml in all the final sperm suspensions. Each sample was halved and the resulting pairs were incubated for 0 to 240 minutes under the same conditions after mixing one sample of each pair with different concentrations of KCl (10^{-9} M up to 1M) (Sigma). The other sample of each pair was used as control sample. At the end of the incubation period (0 to 240 min) both samples of each pair were tested for assessment of sperm motility (% motile spermatozoa) curvilinear velocity, straight line velocity, linearity and amplitude of lateral head displacement employing the CASA system (Hamilton and Thorn). The technique reduces the subjective nature of some aspects of seminal fluid analysis while providing automated measurements of sperm movement characteristics. It allows semen samples to be evaluated by video microscopy. The system stores 20-30 consecutive video frames at a rate of 25-30 frames per second. These stored images are then processed to remove any statistic objects such as debris and immotile sperm. Student's t-test for non paired data was used for statistical analysis of the differences in the sperm motility (% motile spermatozoa) curvilinear velocity, straight line velocity, linearity and amplitude of lateral head displacement, between control samples and samples incubated with KCl. A probability less

than 0.05 was considered to be statistical significant. All the data was expressed as mean value ± standard deviation.

RESULTS AND DISCUSSION

Statistical analysis of the results showed that KCl did not influence significantly any on the above parameters of sperm motility, under the experimental conditions adopted. These results indicate the functional absence of potential dependent channels and suggest that the use o calcium channel blockers, such as verapamil anc dihydropiridines, do not affect the physiology o the mature male gamete.

REFERENCES

World Health Organization, WHO: Laboratory Manual fo the examination of human semen and semen-cervica mucus interaction, 4th ed., Cambridge: The Press syndicate of the University of Cambridge, 1999