Trequinsin HCl: A novel specific human sperm motility inducer identified by High-Throughput Screening using intracellular Ca^{2+} signalling as a surrogate physiological response

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Introduction: Sperm dysfunction is the commonest cause of infertility\(^1\). Despite the unmet need for therapeutic options for male factor subfertility, there is limited progress in our understanding of sperm physiology that controls human sperm function thus, hampering novel drug design/discovery. However, it is acknowledged that intracellular Ca^{2+} signalling is pivotal in human sperm functioning\(^2\) (such as acrosome reaction, hyperactivation, and chemotaxis). Several compounds are known to induce human sperm motility such as methylxanthines, pentoxifylline, caffeine, and IBMX. However, these drugs stimulate human sperm motility at high concentrations (1-10 mM), thus leading into premature acrosome reaction. Therefore, there is a need for specific human sperm motility inducer that can be used at clinically relevant concentrations. Validated high-throughput screening of compounds from University of Dundee Drug Discovery Unit\(^3\) has identified Trequinsin HCl, an ultrapotent phosphodiesterase type 3 inhibitor, to induce significant increase in intracellular [Ca^{2+}] in human spermatozoa and human sperm motility without stimulating acrosome reaction.

Methods: Semen samples from healthy volunteer donors and patients attending Ninewells Assisted Conception Unit (ethical approval 08/S1402/6) were prepared by percoll discontinuous density gradient (40/80). Motility parameters, following treatment with Trequinsin, were evaluated in-vitro using Hamilton Thorne CASA system (Ceros v12). Effect of Trequinsin on acrosome reaction was evaluated by flow cytometry (FACS).

Results

**Effect of 10μM Trequinsin on 40% fraction sperm under non-capacitating conditions.**

![Figure 1](image1.png)

**Effect of 10μM Trequinsin on 40% fraction sperm under capacitating conditions.**

![Figure 2](image2.png)

**Effect of 10μM Trequinsin on 80% fraction donor sperm under non-capacitating and capacitating conditions.**

![Figure 3](image3.png)

**Effect of 10μM Trequinsin on 40% fraction sperm motility of failed fertilisation ICSI patient.**

![Figure 4](image4.png)

**Effect of 10μM Trequinsin on acrosome reaction in 80% capacitated donor sperm.**

![Figure 5](image5.png)

Conclusion: Trequinsin HCl is an effective drug in stimulating human sperm motility at 10μM concentration under both capacitating and non-capacitating conditions without inducing acrosome reaction. These data support further basic and clinical trials investigating the effect of Trequinsin on other aspects of sperm function as well as its therapeutical value in hope to improve live birth rates.