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Opinion Article

Importance of the assessment of intracellular Ca^{2+} level as diagnostic tool of dysfunctional sperm ☆

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ABSTRACT

Sperm functions are an important factor for fertility/pregnancy to be achieved. Sperm dysfunction is the most common cause of male infertility. The best option to help couples with such male factor to achieve a pregnancy, is using Assisted Reproductive Technology (ART), without defining the underlying cause of sperm dysfunction or male-factor infertility in general at the cellular and molecular levels. Thus, the limited success of ART in a proportion of male infertility cases is unsurprising. Ca^{2+} signalling plays a fundamental role in the regulation of sperm function. Interestingly, it appears that the potential exists to diagnose abnormalities in the Ca^{2+} channels that underlie sperm dysfunction. This raises the potential for future drug discovery to try to correct this defect by augmenting Ca^{2+} signalling such as Ca^{2+} store mobilisation or activating CatSper, as possible rational treatments for sperm dysfunction that may temporarily increase the capacity to interact with the egg. Such a pharmacological agent may provide a useful way of increasing the effectiveness of IUI or IVF over conventional IUI and IVF procedures.

Introduction

Male-factor infertility that manifests as aberrant spermatogenesis and/or inadequate sperm function is a significant and increasing global problem, implicated in approximately 40% of subfertility cases [1–3]. An epidemiological study has suggested that sperm dysfunction is the most common cause of male infertility [4]. Currently, the main option for treating sperm dysfunction or male-factor infertility in general is assisted reproductive technology (ART), without defining the underlying cause of sperm dysfunction or male-factor infertility in general at the cellular and molecular levels. Thus, the limited success of ART in a proportion of male infertility cases is unsurprising [5–7].

Sperm dysfunction cannot be detected by the standard descriptive semen analysis, which is recognised as a relatively insensitive tool for predicting the fertilising potential of sperm, except in extreme cases. Interestingly, men can produce dysfunctional sperm even when their semen parameters (sperm concentration and motility) are normal. Hull and colleagues (1985) found the presence of sperm defects diagnosed by failure of sperm to penetrate the cervical mucus in a third of sub-fertile men with normal semen analyses [4]. Men with unexplained infertility with a normal semen analysis were shown to have a higher proportion of sperm dysfunction, such as low hyperactivated motility and a reduced ability of the sperm to penetrate the zona pellucida compared to fertile men. [8]. A study by Alasmari and colleagues (2013) on Ca^{2+} store mobilisation and hyperactivation (HA) in samples from sub fertile men similarly found defects in these processes occurred in samples with normal sperm concentration and motility [9,10]. Moreover, number of/ studies have reported that a significant number of men with unexplained infertility have a male contributing factor, such as high sperm-DNA damage, which is another defect that is not detected by the standard descriptive semen analysis [11–13]. These studies provide a greater understanding with respect to what sperm function assays could add to diagnostic andrology in clinical practice. More advanced sperm-function tests, rather than simple semen analyses, may be justified to help in the accurate diagnosis of male infertility and in decision-making regarding the most appropriate fertility treatments, including intrauterine insemination (IUI), IVF and intracytoplasmic sperm injection (ICSI). Diagnosis of sperm defects could open the door to the invention of novel ways to correct these defects in conjunction with IUI and IVF treatment cycles, which could consequently maximize success rates and benefit a higher proportion of infertile couples.

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Role of sperm functions in ART

The interests of andrology researchers have been directed toward assessing the sophisticated functional aspects of sperm that are essential for both passage through the female tract and gamete interactions, in relation to a sperm sample's fertilising potential. Several studies have reported that the ability of sperm to migrate into the cervical mucus could be used to assess its fertilising potential *in vivo* [14] and *in vitro* [15,16]. Moreover, the measurement of hyperactivation is reported as a useful functional assay to diagnose the fertilising potential of human sperm [17]. The other functional assays to assess gamete interactions are the sperm-zona pellucida binding test and induction of acrosome reactions, which both show a high predictive value for fertilisation rates *in vitro* [18,19,1]. More importantly, it has been reported that sperm function is associated with the quality of the sperm genome apparatus, as dysfunctional cells (low HA and decreased oocyte-penetrating ability) show high DNA fragmentation [20] and abnormal sperm morphology [21]. It is generally accepted that sperm DNA damage (poor sperm genome) is related to poor outcomes following ART, including impaired embryo development, miscarriage and birth defects [22,23,3]. Diagnosis of sperm dysfunction is likely to be a key component in improving ART outcomes, either by a selection of sperm with normal sperm functions or treatment of the underlying defects of dysfunctional sperm. Since it has been reported that sperm with normal morphology exhibit high sperm function [21], therefore a technique of selection of motile spermatozoa with morphologically normal nuclei could help to select functional cells and good-quality DNA-intact sperm, which potentially improve embryo quality and its ability to implant [24–27].

Although the role of sperm functions in fertilisation success is reported in the literature, so far no real clinical progress has been made in the involvement of sperm-function tests in diagnostic andrology. Accordingly, sperm dysfunction is still not diagnosed and is indeed ignored in the diagnosis. To implement such tests on the clinical pathways, these assays must be "robust, cheap, easy to use and clinically useful" [28,7].

Calcium signalling and sperm dysfunction

Ca^{2+} signalling plays a fundamental role in the regulation of sperm function. Interestingly, Ca^{2+} signalling is highly compartmentalized in human sperm, as each source of the signalling has evolved a specific function [29]. Human spermatozoa have evolved the ability to perform specific functions depending on the origin of the Ca^{2+} signal. A study by Alasmari and colleagues (2013) demonstrated that Ca^{2+} signals generated by CatSper activation in the principal piece of the flagellum and the mobilisation of stored Ca^{2+} at the base of the flagellum exert different effects on the flagellar activity of human sperm; Ca^{2+} influx through CatSper channels in the principal piece enhances sperm penetration into the viscous medium, whilst Ca^{2+} released from an internal store at the base of the flagellum strongly induces sperm hyperactivated motility [30].

Interestingly, these calcium signals are defective in cases of sperm dysfunction. Several clinical studies have observed a low responsiveness of human spermatozoa to progesterone (in terms of intracellular Ca^{2+} concentrations $[Ca^{2+}]_i$ and enhancement of sperm function, such as motility and acrosome reaction) from infertile [31,19], oligozoospermic [32], teratozoospermic [33] and asthenzoospermic patients [34]. Earlier genetic studies have indicated that mutation in human CatSper channels are notable in cases of male infertility [35,36]. Shu et al. (2015) specify that defect or downregulation of CatSper gene has an important role in the etiology of idiopathic asthenzoospermia [37]. Similarly, other studies demonstrated that level of CatSper1 and CatSper2 were significantly lower in spermatozoa of oligoasthenzoospermic patients compared to normozoospermic samples [38,39]. However, these cases involved semen that had already demonstrated abnormalities in spermatogenesis (abnormal semen profiles) but did not address abnormalities in signal-mediated sperm dysfunction in apparently normal semen.

A study by Alasmari et al. (2013) demonstrated the occurrence of poor $[Ca^{2+}]_i$ responses to agonists of the mobilisation of stored Ca^{2+} (4-AP), even in patients with normal semen profiles who are undergoing IVF treatment. More significantly, these defects in Ca^{2+} store-mediated poor sperm hyperactivation have been shown to be a novel cause of sperm dysfunction, which reduces IVF success in a small proportion of cases. Furthermore, this study tried to understand the contribution of calcium signalling to fertilisation success and the causes of sperm dysfunction and its place on the infertility spectrum, as it showed the relationship between responsiveness of human sperm to 4-AP (the mobilising agent of neck/midpiece Ca^{2+} stores) or progesterone (CatSper agonist) and the IVF fertilisation rate, which confirmed the functional significance of the neck/midpiece Ca^{2+} store and CatSper-dependent sperm behaviour in male fertility [9]. Experimental study showed that mice female gametes (cumulus cells) express as well a regulator protein (CRISP1) of CatSper, a protein essential for modulating sperm motility to penetrate the cumulus cells. Moreover, cumulus-oocyte complexes from CRISP1 knockout females are unable to be fertilized due to failure of sperm to penetrate the egg vestments. This suggests the importance of CatSper for sperm progression through the cumulus layers to achieve fertilisation [40].

Therefore, these studies indicate that CatSper channels and store mobilisation appear to be important potential targets for pharmacological interventions in sperm function, either by improving the sperm's fertilising potential (for IUI and IVF) or by reducing its fertilising capacity (for male contraception). Ion channels are important therapeutic targets for numerous disorders, for example, neurological and cardiovascular disorders [41].

Implications of the diagnosis of sperm dysfunction on treatment of male infertility

Interestingly, it appears that the potential exists to diagnose abnormalities in the Ca^{2+} stores or CatSpers that underlie sperm dysfunction. This raises the potential for future drug discovery to try to correct this defect by augmenting Ca^{2+} store mobilisation or activating CatSper, as possible rational treatments for sperm dysfunction that may temporarily increase the capacity to interact with the egg. Such a pharmacological agent may provide a useful way of increasing the effectiveness of IUI or IVF over conventional IUI and IVF procedures. For example, if patients were diagnosed with defective CatSper function by exhibiting poor viscous substrate-penetrating ability [30], a novel CatSper-stimulating drug could restore/improve sperm function and might exert a positive effect on the dynamics of sperm transport through the mucus of the female tract following IUI for proportion of patients. Moreover, as some patients with defective Ca^{2+} stores exhibit low IVF fertilisation rates [9], the development of effective and safe drugs designed to act as Ca^{2+} store stimulants may be beneficial for this subgroup.

Importantly, a study by William et al. (2015) found cases with the complete absence of CatSper current in response to progesterone, associated with the failure of sperm to penetrate the viscous media and leading to complete failure of fertilisation even when semen characteristics were normal. This suggests that Cat-

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