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Oocyte activation, phospholipase C zeta and human infertility

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BACKGROUND: Mammalian oocytes are activated by intracellular calcium (Ca^{2+}) oscillations following gamete fusion. Recent evidence implicates a sperm-specific phospholipase C zeta, PLCζ, which is introduced into the oocyte following membrane fusion, as the responsible factor. This review summarizes the current understanding of human oocyte activation failure and describes recent discoveries linking certain cases of male infertility with defects in PLCζ expression and activity. How these latest findings may influence future diagnosis and treatment options are also discussed.

METHODS: Systematic literature searches were performed using PubMed, ISI-Web of Knowledge and The Cochrane Library. We also scrutinized material from the United Nations and World Health Organization databases (UNWHO) and the Human Fertilization and Embryology Authority (HFEA).

RESULTS AND CONCLUSIONS: Although ICSI results in average fertilization rates of 70%, complete or virtually complete fertilization failure still occurs in 1–5% of ICSI cycles. While oocyte activation failure can, in some cases, be overcome by artificial oocyte activators such as calcium ionophores, a more physiological oocyte activation agent might release Ca^{2+} within the oocyte in a more efficient and controlled manner. As PLCζ is now widely considered to be the physiological agent responsible for activating mammalian oocytes, it represents both a novel diagnostic biomarker of oocyte activation capability and a possible mode of treatment for certain types of male infertility.

Key words: human infertility / oocyte activation / sperm / phospholipase C zeta / calcium

Introduction

Infertility affects both genders in very different ways (Evens, 2004). Although it is difficult to compile accurate data for the incidence of global infertility, it is believed that infertility affects 70 million couples worldwide, the majority of whom reside in developing countries (Ombelet *et al.*, 2008). The incidence of global infertility is now estimated to affect one in seven couples (Evens, 2004;

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Boivin et al., 2007; Ombelet et al., 2008; Ledger, 2008; McVeigh et al., 2008).

Since infertility represents a major physiological and psychological problem to a growing proportion of the population, governments worldwide are investing heavily in assisted reproductive technology (ART) which has led to significant improvements in our understanding of male/female reproductive systems, gamete preservation and gamete manipulation (Elder and Dale, 2001; Cohen et al., 2005). ART now accounts for 7% of all births in some developed countries (Nasr-Esfahani et al., 2009). Worldwide, ~1 million ART treatments are performed each year and over 8 million ART babies have been born worldwide (ICMART, 2009).

The most widely known example of ART is *in vitro* fertilization (IVF). Initially developed to treat women with tubal infertility, IVF is now an established treatment for a wide range of infertilities (McVeigh et al., 2008). Over 30 years after the birth of the world's first IVF baby, more than 12 000 births in the UK arise each year as a result of IVF, accounting for 1.4% of all births in the UK (HFEA register, 2007). A key technique in IVF is intracytoplasmic sperm injection (ICSI), whereby a sperm is microinjected directly into the oocyte cytosol. ICSI is predominantly used to treat male factor infertility following failure of conventional IVF, and is a highly successful technique that, on average, results in normal fertilization in 70% of cases (Nasr-Esfahani et al., 2009).

Current ART methods facilitate the treatment of most forms of infertility (Fig. 1a and b). For example, infertile females suffering from abnormal ovulation can be treated by *in vitro* maturation (IVM), a method used to mature oocytes *in vitro* in preparation for IVF (Cohen et al., 2005). Similarly, ICSI can rectify a number of male factor problems, such as oligospermia (low sperm count), transcending the need for a normal sperm count/motility (Mangoli et al., 2008; McVeigh et al., 2008). Although ART plays a critical role in reducing infertility, there are still groups of infertile couples for which ART has not yet proven successful. Of particular outstanding concern are infertile males whose sperm are unable to activate oocytes, even following ICSI, or males exhibiting unexplained (idiopathic) male factor infertility (Mangoli et al., 2008; Nasr-Esfahani et al., 2009).

Current data suggests that the incidence of 'subfertility' affects 1 in 15 men, with sperm defects being the most common cause (Publicover et al., 2007). However, there is much debate surrounding the specific causative factors of unexplained male infertility, most particularly in cases of ICSI failure. It is estimated that male factor infertility contributes to 35–40% of infertility in couples (Forti and Krausz, 1998; Inhorn, 2003; Boivin et al., 2007; Kumtepe et al., 2009), depending upon the biogeographical region studied. Although male factor infertility can arise in a number of ways (Fig. 1b; Bourne et al., 1995a, b; Elliot and Cooke, 1997; Evens, 2004; Meacham et al., 2007; McVeigh et al., 2008; Kumtepe et al., 2009), it is widely accepted that genetic causes are of most concern. Evidence is mounting to suggest that abnormalities in testicular gene expression may underlie many instances of idiopathic male infertility (Coward et al., 2007). One such example is the azoospermia factor Y chromosome gene region, which is prone to mutation (Nagafuchi et al., 1993; Vogt et al., 1995). Other Y chromosome gene regions, such as RBM (RNA-binding-motif) and DAZLA (deleted in azoospermia) are implicated in spermatogenesis and the structural formation of sperm (Elliot and Cooke, 1997; Ruggiu et al., 1997; Ambasudhan et al., 2003). More

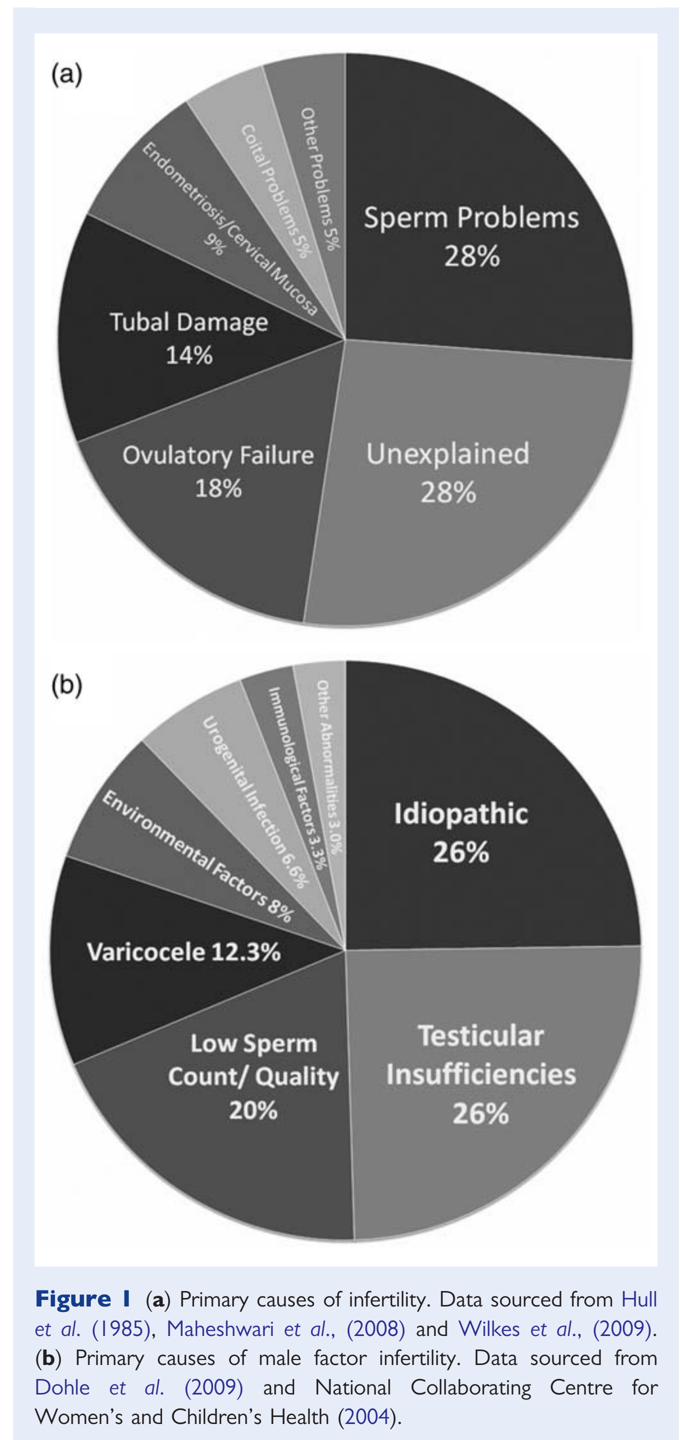

Figure 1 (**a**) Primary causes of infertility. Data sourced from Hull et al. (1985), Maheshwari et al., (2008) and Wilkes et al., (2009). (**b**) Primary causes of male factor infertility. Data sourced from Dohle et al. (2009) and National Collaborating Centre for Women's and Children's Health (2004).

recently, mutations in CATSPER1 have been found to cause infertility (Avenarius et al., 2009). Further examples include AKAP3 and AKAP4, deletion of which leads to sperm immotility and dysplasia of the fibrous sheath surrounding the axenome (Chemes et al., 1987; Turner et al., 2001; Matzuk and Lamb, 2008). Mouse models have increasingly highlighted hundreds of candidate genes which may be involved in human male infertility (Matzuk and Lamb, 2008; Poongothai et al., 2009). Idiopathic male infertility may be the result of multiple genetic defects disrupting spermatogenesis and testicular gene expression, indicating that ART interventions such as ICSI could

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potentially transfer paternal fertility problems from father to son (Sasaki et al., 2000).

A noted phenomenon of male infertility is the failure of some oocytes to activate following ICSI (Mahutte and Arici, 2003; Heindryckx et al., 2005). One explanation for this is globozoospermia, a condition affecting 0.1% of infertile men (Dam et al., 2007) in which acrosome formation and oocyte activation capacity are abnormal due to deformed morphology of the sperm. However, there are also examples where morphologically normal sperm fail to activate the oocyte. Recent studies have linked globozoospermia and these other cases with oocyte activation failure as being apparently due to aberrant expression, localization and protein structure of PLCζ (Yoon et al., 2008; Heytens et al., 2009).

In this review, our aim is to discuss the current understanding of oocyte activation failure, and associated treatment options, focusing specifically upon the role played by the sperm-derived oocyte activation factor, PLCζ (Saunders et al., 2002; Swann et al., 2004, 2006; Saunders et al., 2007; Parrington et al., 2007). We will also discuss recent discoveries linking PLCζ to characterized states of infertility (Yoon et al., 2008; Heytens et al., 2009) and how these findings might assist diagnosis and treatment in future.

Methods

To generate this review, we carried out a systematic literature search using PubMed, ISI-Web of Knowledge and The Cochrane Library. Search terms included phospholipase (PLC), phospholipase C zeta/PLCzeta/PLCζ, fertilization, oocyte activation, intracytoplasmic sperm injection/ICSI, globozoospermia, male factor infertility, oocyte activation failure and artificial oocyte activation (AOA). We also scrutinized electronic material from the United Nations and World Health Organization databases (UNWHO) and the Human Fertilization and Embryology Authority (HFEA).

Results

Identification of PLCζ as the oocyte activation factor in mammals

Mammalian oocytes become arrested at the metaphase of the second meiotic division (MII), following the exclusion of the first polar body (Jones, 2005, 2007). One of the most important biological processes involves the release of this arrest to allow development to proceed, a process termed 'oocyte activation'. Activation initiates release from meiotic arrest, cortical granule exocytosis, progression of the cell cycle, pronuclear formation, maternal mRNA recruitment and embryonic gene expression, and involves repeated oscillations of free cytosolic Ca^{2+} (Kline and Kline, 1992; Swann and Ozil, 1994; Miyazaki and Ito, 2006; Publicover et al., 2007; Swann and Yu, 2008). In contrast, egg activation in non-mammalian species such as sea urchins and frogs, is triggered by a single Ca^{2+} transient (Whitaker, 2006).

The first evidence for the importance of Ca^{2+} signals in egg and oocyte activation came from the application of Ca^{2+} dyes to eggs and oocytes from a wide range of species, associating increases in cytosolic Ca^{2+} with initiation of activation and embryogenesis (Ducibella et al., 2006). Microinjections of Ca^{2+} ions alone are sufficient to trigger embryo development up to the blastocyst stage in mice (Fulton and Whittingham, 1978; Swann and Yu, 2008). Other studies have shown that the temporal pattern of Ca^{2+} oscillations is largely species-specific, with different species possessing specific patterns of amplitude, duration and frequency over time (Miyazaki et al., 1993; Jones et al., 1998; Kyozuka et al., 1998; Ducibella et al., 2002, 2006).

Although it is now generally acknowledged that Ca^{2+} oscillations within the mammalian oocyte are a direct result of inositol triphosphate (IP_3) mediated Ca^{2+} release (Parrington, 2001; Swann et al., 2004, 2006; Whitaker, 2006; Saunders et al., 2007; Parrington et al., 2007; Swann and Yu, 2008), the precise mechanism underlying these oscillations has been unclear and the subject of intense debate, specifically in relation to the relative roles played by the sperm and oocyte. A major breakthrough in our understanding came with the discovery of a soluble 'sperm factor', a sperm-derived phospholipase with distinctive properties (Jones et al., 1998; Parrington et al., 1999; Jones et al., 2000; Rice et al., 2000; Parrington et al., 2000, 2002), finally identified as PLC zeta (PLCζ) (Saunders et al., 2002). Numerous studies provide evidence for PLCζ being the physiological agent of oocyte activation. Injection of both recombinant PLCζ RNA and protein into mouse oocytes results in the initiation of Ca^{2+} oscillations, similar to those seen at fertilization, and embryonic development to the blastocyst stage (Saunders et al., 2002; Cox et al., 2002; Kouchi et al., 2005). Immuno-depletion of PLCζ from sperm extracts suppresses Ca^{2+} releasing ability (Saunders et al., 2002), whereas sperm fractionation studies have indicated that the presence of PLCζ in sperm correlates with the sperm's ability to induce Ca^{2+} oscillations in the oocyte (Fujimoto et al., 2004; Kurokawa et al., 2005). Furthermore, RNA interference (RNAi) experiments have produced transgenic mice with significantly reduced expression of PLCζ in the testis (Knott et al., 2005). Fertilization by sperm from these animals is characterized by a premature cessation of Ca^{2+} oscillations within the oocyte. PLCζ appears to play a similar role during fertilization in non-mammalian species such as the chicken (Coward et al., 2005), medaka fish (Ito et al., 2008b) and quail (Mizushima et al., 2009), suggesting the existence of a mechanism common to all vertebrates.

Studies in mice have concluded that PLCζ mRNA is first detectable in spermatids as opposed to testicular cells depleted of spermatids (Saunders et al., 2002). More systematic RT–PCR studies of PLCζ mRNA expression during spermatogenesis in the pig and quail have indicated that PLCζ mRNA is most likely translated in elongating spermatids (Yoneda et al., 2006; Mizushima et al., 2009), whereas northern blot analyses of postnatal hamsters has shown that PLCζ mRNA is present as early as Day 17 (Young et al., 2009), when meiotic spermatocytes first appear (Golan et al., 2000).

Although the general consensus is now that PLCζ is probably the trigger of development in mammals (Parrington et al., 2007), recent studies have demonstrated possible candidates for sperm factors apart from PLCζ, which are able to induce meiotic progression or the typical pattern of Ca^{2+} release in different species. Harada et al. (2007) identified a 45 kDa protein, citrate synthase, as the major component responsible for oocyte activation in the newt *Cynops pyrrhogaster*. Injection of Xenopus citrate synthase mRNA and porcine citrate synthase induced efficient activation of newt oocytes. However, the detailed molecular mechanism or pathway involved remains to be addressed. Wu et al. (2007) reported another possible candidate for the sperm factor, post-acrosomal sheath WW domain-binding protein (PAWP), which resides in the post-acrosomal sheath region of the perinuclear theca in bovine sperm and other mammalian species. Microinjection of recombinant PAWP into MII oocytes provokes pronuclear formation in different mammalian species (porcine, bovine, macaque). A recent study confirmed the potential of recombinant PAWP to induce Ca^{2+} release using Xenopus oocytes (Aarabi et al., 2010). Although the molecular mechanisms underlying the precise function of PAWP are currently unknown, it was suggested that both PAWP and PLCζ posses a double role in the oocyte activation mechanism or alternatively, that the PAWP-mediated signalling pathway may act upstream or downstream of Ca^{2+} signalling (Wu et al., 2007; Aarabi et al., 2010).

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The oocyte itself also appears to play a pivotal role in the activation mechanism (Tesarik *et al*., 2002; Heindryckx *et al*., 2005; 2007), the exact details of which are yet to be properly understood (Ajduk *et al*., 2008). The ability to produce appropriate Ca^{2+} oscillations is acquired following successful oocyte maturation and involves various cytoplasmic changes. Evidence for this includes the fact that fertilized immature mouse oocytes generate fewer Ca^{2+} transients of lower amplitude than do oocytes fertilized at MII (Cheung *et al*., 2000). Eppig *et al*. (1994) suggested that cytoplasmic maturation was not fully complete in oocytes from younger mice. During oocyte maturation, the Ca^{2+} release mechanism is installed between prophase I (germinal vesicle stage) and MII, coupled with a variety of cytoplasmic changes including reorganization of the endoplasmatic reticulum, an increase in the number and sensitivity of IP_3 receptors, and an increase in Ca^{2+} concentration (Fissore *et al*., 2002; Ajduk *et al*., 2008; Swain and Pool, 2008). Recent findings suggest that the distribution and function of mitochondria within the oocyte also plays an instrumental role through the endoplasmic reticulum and IP_3 mediated Ca^{2+}-signalling (Van Blerkom *et al*., 2002; Dumollard *et al*., 2006).

PLCζ as a fundamental agent of oocyte activation

Many questions still prevail concerning the PLCζ mechanism of action (Fig. 2), cellular localization and potential role in male factor infertility. PLCζ exhibits a typical PLC domain structure (Saunders *et al*., 2002) and has the closest homology with PLCδ isoforms (Katan, 1998; Rebecchi and Pentyala, 2000), particularly with PLCδ1 (Swann *et al*., 2006). PLCζ possesses characteristic X and Y catalytic domains which form the active site common to all PLCs (Rebecchi and Pentyala, 2000; Swann *et al*., 2006), a C2 domain, and a set of EF hands. One major difference is the absence of a PH domain, making PLCζ the smallest known mammalian PLC (Rebecchi and Pentyala, 2000; Saunders *et al*., 2002). All active site residues are conserved, or conservatively replaced, and their mutagenesis leads to the loss of Ca^{2+} induction ability, confirming that as with PLCδ1, the active site of PLCζ is responsible for targeting Phosphatidylinositol 4,5-bisphosphate (PIP_2) to cause IP_3 mediated Ca^{2+} release (Swann *et al*., 2006).

PLCζ is also distinctive compared with other PLC isoforms in its high sensitivity to Ca^{2+} (Rebecchi and Pentyala, 2000; Kouchi *et al*., 2004), which may explain why PLCζ is much more effective than other PLCs at causing IP_3 production and Ca^{2+} release in the oocyte cytoplasm (Swann *et al*., 2006). Mammalian PLCζs have four EF-hand motifs at their N-termini, which appear to play an important structural role in PIP_2 targeting and enzyme activity (Fujimoto *et al*., 2004; Kouchi *et al*., 2004; Yoda *et al*., 2004; Kurokawa *et al*., 2005; Nomikos *et al*., 2005; Swann *et al*., 2006; Nakanishi *et al*., 2008).

Without a PH domain, it remains unclear how PLCζ targets its membrane-bound substrate PIP_2, since in other PLCs the PH domain serves to anchor the enzyme to either specific plasma membrane proteins, as is the case for the β, γ and ε isoforms of PLCs, or directly to PIP_2 as is the case in PLCδ1 (Rebecchi and Pentyala, 2000; Rhee, 2001). Intriguingly, Lee *et al*. (2004) suggested that the PH domain is not integral to the membrane localizing ability of PLCδ4. Although the C2 domain is considered a regulatory domain, it is possible that this domain may aid PLCζ in targeting membrane-bound PIP_2 (Swann *et al*., 2006). There is also evidence to suggest that the C2 domain is required for the ability of PLCζ to initiate oocyte activation (Nomikos *et al*., 2005; Swann *et al*., 2006).

The other non-catalytic domain of PLCζ that may regulate its activity is the segment between the X and Y catalytic domains, the X–Y linker (Swann *et al*., 2006; Nomikos *et al*., 2007). The proximity of this apparently unstructured cluster of residues to the active site indicates some potential involvement in regulating catalytic activity, or PIP_2 binding (Swann *et al*., 2006; Nomikos *et al*., 2007). Evidence suggests that PLCζ remains functional following proteolytic cleavage at the X–Y linker, although data also indicate that these fragments can reform complexes to retain activity (Kurokawa *et al*., 2007). Intriguingly, Hicks *et al*. (2008) showed that the X–Y linker in most, if not all, PLC isoforms plays an auto-inhibitory role, the deletion of which results in elevated activity. Indeed, proteolytic cleavage at the X–Y linker is thought to be required in order for PLCζ to be able to bind to the membrane and act upon PIP_2 (Saunders *et al*., 2007), raising questions as to the involvement of the X–Y linker in PIP_2 binding.

Following fertilization, oocyte Ca^{2+} oscillations cease at the time of pronuclei formation (Marangos *et al*., 2003) with subsequent oscillations then being observed in mouse zygotes during mitosis (Carroll, 2001; Marangos *et al*., 2003). One possible explanation of such cell-cycle dependant termination and resumption, is that PLCζ localizes to the pronuclei during interphase due to a nuclear localization signal (NLS), resulting in the

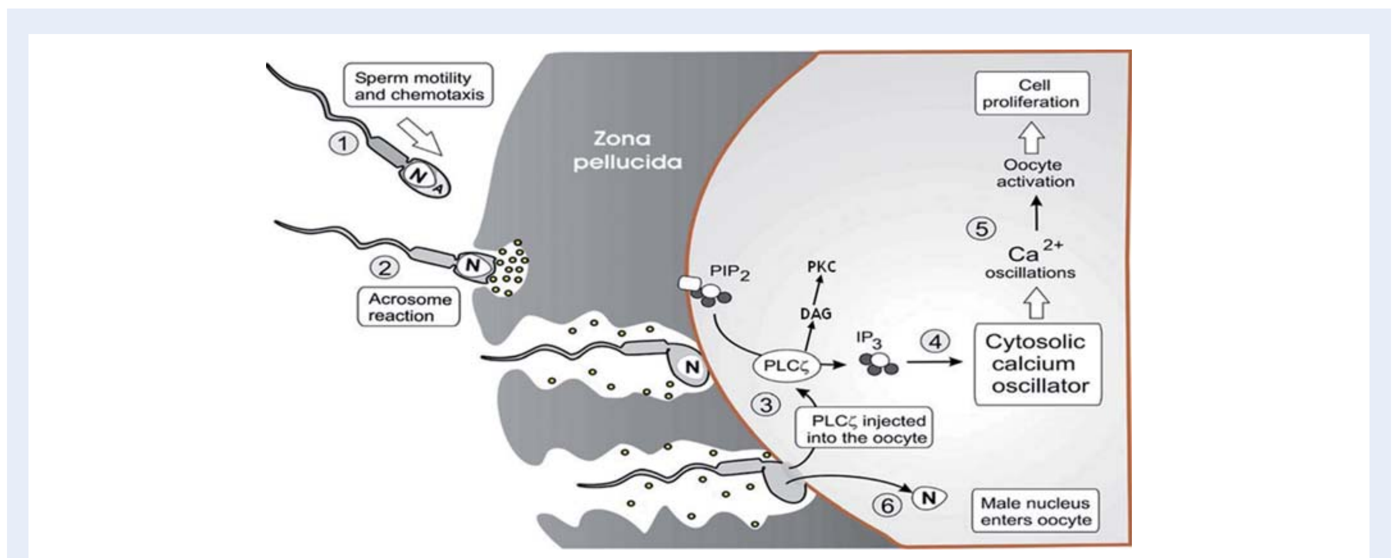

Figure 2 Signalling pathway of PLCζ. Reproduced from Berridge (2009) with permission.

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cessation of Ca^{2+} oscillations which resume following pronuclear envelope breakdown during the fertilized oocyte's entry into mitosis (Deguchi et al., 2000; Larman et al., 2004; Yoda et al., 2004; Sone et al., 2005; Kuroda et al., 2006; Swann et al., 2006; Ito et al., 2008a, b). It remains possible that there is also a role for PLCζ within the nucleus itself. However, human PLCζ does not translocate to the pronucleus when injected into mouse oocytes (Ito et al., 2008a, b), thus the relevance of this phenomenon remains less clear for human fertilization.

Another important issue is the pattern of localization of PLCζ in the sperm. Identification of PLCζ's location in the sperm could help confirm its functional role in sperm as well as provide a benchmark to study the pattern of localization of PLCζ in infertile compared with fertile men. Initial immunofluorescent studies in rodents suggested that in mouse sperm, PLCζ protein locates to the perinuclear theca of the sperm head (Fujimoto et al., 2004), the expected location for the oocyte activation factor (Kimura et al., 1998; Perry et al., 1999; Sutovsky et al., 2003; Swann et al., 2006). Subsequent studies indicated that PLCζ was localized to post-acrosomal and equatorial regions in non-capacitated mouse and bull sperm, respectively (Yoon and Fissore, 2007). In contrast to these findings, a study of PLCζ localization in mice and hamster sperm during capacitation and the acrosome reaction indicated that PLCζ was most prominent in the acrosomal region in uncapacitated sperm of these species, with a post-acrosomal localization being revealed following capacitation (Young et al., 2009). It remains to be shown whether PLCζ carries out other roles besides oocyte activation, such as the acrosome reaction.

In non-capacitated human sperm, PLCζ is predominantly localized to the equatorial region of the sperm head (Grasa et al., 2008), and this pattern of localization is maintained during capacitation and the acrosome reaction. This location would be an ideal one for an oocyte activation factor, since this region would be one the first to be exposed to the oocyte cytoplasm following the acrosome reaction and gamete fusion, thus facilitating easy passage of the protein into the oocyte. However, as well as this predominantly equatorial pattern of localization, PLCζ has also been detected to a lesser extent in acrosomal and post-acrosomal regions of human sperm. The fact that such variability in PLCζ localization is present in ejaculates from individual fertile males, as well as between fertile males, raises the possibility that there may be differences in oocyte activation capabilities between individuals or within an ejaculate, even in the fertile male population (Grasa et al., 2008). Recent studies suggest that PLCζ RNA transcripts are present within human sperm (Platts et al., 2007; Lalancette et al., 2008). It has been suggested that such transcripts could be transcribed during fertilization and sustain a 'long-lived' Ca^{2+} response (Lalancette et al., 2008). However, the functional significance of these PLCζ transcripts, as for all RNA transcripts present in human sperm, remains to be verified.

Oocyte activation failure

ICSI is currently the most efficient ART technique to overcome borderline and severe male infertility, failed IVF or unexplained infertility (The ESHRE Capri Workshop, 2007). Although ICSI results in average fertilization rates of 70% (Heindryckx et al., 2005), complete or virtually complete fertilization failure still occurs in 1–5% of ICSI cycles (Flaherty et al., 1998; Mahutte and Arici, 2003; Yanagida et al., 2008). Incorrect injection of sperm, expulsion of injected spermatozoon from the oocyte or failures of sperm head decondensation are not considered to substantially contribute to fertilization failure after ICSI (Yanagida et al., 2008). A deficiency in the mechanism of oocyte activation is regarded as the principal cause of fertilization failure, or abnormally low fertilization after ICSI, and can reoccur in several cycle attempts (Sousa and Tesarik, 1994; Heindryckx et al., 2008). Additionally, the number of oocytes collected and the presence of motile or vital sperm are both strongly associated with fertilization failure, and have to be taken into account when determining the cause of failed or low fertilization (Yanagida, 2004). There is much clinical interest in investigating the mechanisms underlying oocyte activation failure caused by a sperm- or oocyte-related deficiency. It is of utmost importance to distinguish these two possible causes, primarily to better inform patients about possible transmission to their progeny, and to better understand underlying mechanisms in order to develop remedial treatments. Apart from failure of meiotic progression of the human MII oocyte, sperm centrosomal dysfunction and concomitant lack of sperm aster formation may preclude the close opposition of the pronuclei required for syngamy, leading to pronuclear arrest of human zygotes (Van Blerkom, 1996; Rawe et al., 2002, 2008). Cases of the latter instance can also be considered clinically as failed fertilization after ICSI, but should be distinguished from activation failure and MII arrest.

A diagnostic Mouse Oocyte Activation Test (MOAT) was first used to examine the activation capacity of sperm from a globozoospermic infertile patient by injection into mouse oocytes (Rybouchkin et al., 1996), and was subsequently employed in larger populations of patients with a history of failed ICSI or obvious morphological indications such as globozoospermia (Tesarik et al., 2002; Heindryckx et al., 2005, 2008; Heytens et al., 2009). Importantly, this test can indicate sperm-borne activation deficiencies, revealed by an inability to cause pronuclei formation and division into two cells of mouse oocytes. It has been shown that total or partial globozoospermic, extreme oligo-astheno-teratozoospermic and testicular sperm of some patients fail to activate mouse oocytes, while also failing fertilization after ICSI in humans (Tesarik et al., 2002; Heindryckx et al., 2005, 2008; Kyono et al., 2009). Interestingly, even morphologically normal sperm from several patients fail to activate mouse oocytes (Heindryckx et al., 2008; Heytens et al., 2009).

It should be noted that MOAT results, and the study of associated defects in PLCζ, cannot be strictly extrapolated to human oocytes, since the potency of human PLCζ is higher than of mouse PLCζ (Cox et al., 2002), meaning that a slightly reduced activation capacity in human sperm revealed by the MOAT could cause total fertilization failure in human oocytes. Therefore, the MOAT may not be sensitive enough to reveal activation deficiencies in all sperm samples, and more precise diagnostic tests to determine activation capacity are therefore warranted. Other studies have used a heterologous bovine ICSI model to study centrosomal function of human sperm which gave rise to failed ICSI (Nakamura et al., 2001), leading to the finding that, compared with fertile donor sperm, sperm from one globozoospermic patient possessed centrosomal dysfunction in terms of reduced sperm aster formation which could not be restored by artificial activation (Nakamura et al., 2002). However, ionophore treatment in another globozoospermic patient with reduced centrosomal function reported by the same group resulted in a successful pregnancy (Terada et al., 2009). These findings have not been confirmed by other groups, or on other patients. Given the successfully established pregnancy after application of artificial activation in the latter study, the underlying mechanism of failed fertilization is likely due to activation deficiency rather than centrosomal dysfunction.

Artificial activation of mammalian oocytes

AOA can be induced by a wide range of various chemical, mechanical or physical stimuli, which elicit mostly one Ca^{2+} transient in the oocyte (Alberio et al., 2001). Different methods of artificial activation have been used for studies involving somatic cell nuclear transfer, or the creation of parthenogenetic embryos (Machaty, 2006; Brevini and Gandolfi, 2008). The most popular artificial activating agents for human oocytes include calcium ionophores, such as ionomycin and A23187, electrical pulses, and combinations with protein synthesis or kinase inhibitors such as 6-dimethylaminopurine (6-DMAP), or puromycin that blocks the

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re-synthesis of cyclin B or CDK1 activity (Heindryckx *et al*., 2009). These artificial activation agents cause a single prolonged Ca^{2+} rise, but fail to elicit physiological patterns of Ca^{2+} release. Other activating agents, which have been shown to cause multiple transients, include strontium chloride ($SrCl_2$) in mice (Kline and Kline, 1992; Kishikawa *et al*., 1999), phorbol esters (Cuthbertson and Cobbold, 1985) or thimerosal (Fissore *et al*., 1995). These, however, have only been reported to be efficient in limited number of species, are less efficient than ionophores in most species, or can cause meiotic spindle disruption (Alberio *et al*., 2001).

Studies have demonstrated that whereas human oocytes can be successfully artificially activated, parthenogenetic development mostly arrests at the embryo genome activation stage in humans (Brevini and Gandolfi, 2008). Using donated *in vivo* matured MII oocytes, human parthenogenetic blastocyst formation was accomplished using 6-DMAP as an artificial activating agent. However, there was a large variation in the blastocyst development rates (8.6–52%), with not all obtained blastocysts demonstrating a visible inner cell mass (Cibelli *et al*., 2001; Lin *et al*., 2003; Revazova *et al*., 2007; Paffoni *et al*., 2007; Mai *et al*., 2007; de Fried *et al*., 2008; Heindryckx *et al*., 2009). Parthenogenetic blastocyst formation derived from *in vitro* matured GV oocytes using a combined electrical–chemical activation protocol was achieved, but only one blastocyst was obtained (Yu *et al*., 2009).

Several studies in animals have demonstrated that the number and amplitude of artificially induced Ca^{2+} transients not only affects activation efficiency, but also has a profound influence on subsequent embryonic development (Ozil *et al*., 2006), blastocyst quality (Bos-Mikich *et al*., 1997), the implantation potential of rabbit parthenogenotes (Ozil and Huneau, 2001) and mouse zygotes (Ozil *et al*., 2006), and results in altered embryonic gene expression (Ozil *et al*., 2006). The knockdown of PLCζ in a transgenic mouse model showed that a reduced frequency of Ca^{2+} oscillations caused decreased activation rates, impairing the implantation potential (Knott *et al*., 2005). However, it has also been suggested that the sum of elevated Ca^{2+} is most important, rather than a normal pattern of Ca^{2+} oscillations (Ozil *et al*., 2005; Toth *et al*., 2006).

Not all artificial activating agents faithfully mimic the pattern of oscillations caused by PLCζ. In contrast, when cRNA encoding the full-length of PLCζ protein from different species was injected into mouse oocytes, Ca^{2+} oscillations similar to those evoked by sperm were observed (Cox *et al*., 2002; Saunders *et al*., 2002), leading to successful embryonic development up to the blastocyst stage. Rogers *et al*. (2004) demonstrated that the frequency of Ca^{2+} oscillations correlated with the concentration of PLCζ cRNA used. A higher concentration of 10 μg/ml PLCζ cRNA resulted in high-frequency oscillations inducing efficient activation (pronuclei formation). However, only 2 of 10 activated zygotes reached the 2-cell stage, subsequently arresting. Earlier experiments injecting human PLCζ cRNA into mouse oocytes showed that high-frequency Ca^{2+} oscillations give rise to cleavage stage arrest (Cox *et al*., 2002). By decreasing the PLCζ cRNA concentration, development up to the blastocyst stage was achieved using human oocytes which had failed to fertilize (Rogers *et al*., 2004). Furthermore, Yu *et al*. (2008) demonstrated how much PLCζ protein is required for optimal embryonic development by injecting cRNA encoding luciferase-tagged human PLCζ into mouse oocytes and observing subsequent parthenogenetic development, demonstrating that activation can be induced by a wide range of cRNA concentrations, but that successful preimplantation development was critically dependent on a specific narrow window of PLCζ levels.

However, for clinical purposes injection of PLCζ cRNA could be problematic, partly because it could be difficult to limit its oscillation inducing capacity during embryo development, but also because of studies suggesting that mammalian embryos may contain an endogenous reverse transcriptase activity (Spadafora, 2004) that could potentially convert introduced cRNAs into PLCζ cDNA that might then become incorporated into the genome. The use of recombinant PLCζ protein has also been shown to induce equivalent Ca^{2+}-oscillations in mouse oocytes (Kouchi *et al*., 2004). However, many researchers have reported that PLCζ protein appears to be unstable and may not be fully active and a demonstration that PLCζ protein can be used clinically to overcome defects in oocyte activation still remains to be shown.

Clinical use of assisted oocyte activation

Since the first report of AOA (Rybouchkin *et al*., 1997), numerous studies have reported pregnancies after the application of AOA as a treatment for failed or low fertilization after ICSI (for reviews, see Yanagida *et al*., 2008; Nasr-Esfahani *et al*., 2009). Unfortunately, most studies have lacked information about the cause of fertilization failure, for instance from diagnostic tests such as the MOAT. Heindryckx *et al*. (2005, 2008) demonstrated that AOA is highly efficient for couples suffering from low or failed fertilization, with concomitant failure of pregnancy. Fertilization rates were significantly increased after AOA application to a normal level (74%) and successful pregnancies were established in all groups of patients (33%), although patients with extreme OAT-zoospermia showed lower pregnancy rates after AOA (9%). This shows that AOA is capable of initiating artificial Ca^{2+} rises in the oocyte cytoplasm, sufficient to normalize fertilization rates and establish pregnancies in such patients (Heindryckx *et al*., 2005, 2008). A couple with a history of low fertilization (17% in eight cycles, no pregnancy) was successfully treated in a cycle of AOA with $SrCl_2$, resulting in a 100% fertilization rate, with pregnancies from fresh and frozen embryos (Yanagida *et al*., 2006). In a larger set of nine patients, Kyono *et al*. (2008) showed that fertilization and pregnancy rates were significantly increased following the use of $SrCl_2$ (22–65 and 0–40%, respectively). However, no diagnostic tests were used in these studies to reveal sperm related activation deficiencies, and it has yet to be elucidated whether $SrCl_2$ is an efficient alternative for AOA since some groups have failed to induce artificial activation in human oocytes using $SrCl_2$ (Rogers *et al*., 2004; Heindryckx *et al*., 2009).

Although calcium ionophores are commonly used for AOA (Nasr-Esfahani *et al*., 2009), a modified ICSI method has been used to treat failed ICSI, based on the vigorous aspiration of the oocyte cytoplasm, which gives rise to successful pregnancies (Tesarik *et al*., 2002; Ebner *et al*., 2004). However, these findings have not been reproduced. Electrical pulses have been described to overcome fertilization failure in one case report (Yanagida *et al*., 1999), which was recently confirmed in a large-scale study of 71 patients with a history of total fertilization failure (Baltaci *et al*., 2009). In this study, 21 patients underwent routine ICSI without or with concurrent electrical pulses on sibling oocytes, resulting in a fertilization rate of 12 and 62%, respectively; however, no pregnancy was reported. Furthermore, 50 patients all underwent AOA with electrical pulses, resulting in fertilization and pregnancy rates of 48 and 42%, respectively.

The advent of technologies such as ICSI combined with AOA has given much hope for the treatment of conditions such as globozoospermia. The vast majority of cases where routine ICSI has been performed using globozoospermic sperm have had very low success rates in achieving pregnancy (Bourne *et al*., 1995a, b; Liu *et al*., 1995; Trokoudes *et al*., 1995; Battaglia *et al*., 1997; Kilani *et al*., 1998, 2004). However, there have since been a number of cases demonstrating that ICSI combined with AOA greatly increases the success rate of fertilization and subsequent pregnancy (Rybouchkin *et al*., 1997; Kim *et al*., 2001; Tesarik *et al*., 2002; Heindryckx *et al*., 2005, 2008; Dirican *et al*., 2008; Tejara *et al*., 2008; Kyono *et al*., 2009; Taylor *et al*., 2010). Since

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an erroneous Ca^{2+} pattern may cause impaired embryonic developmental potential in animal models (Ozil *et al*., 2006), it is also possible that other infertile patients showing embryo arrest, low embryo quality and even recurrent implantation failure might also benefit from some form of AOA.

Links between PLCζ defects and oocyte activation failure

Given the proposed role of PLCζ in oocyte activation, it is possible that abnormal forms or aberrant function of PLCζ may be the underlying

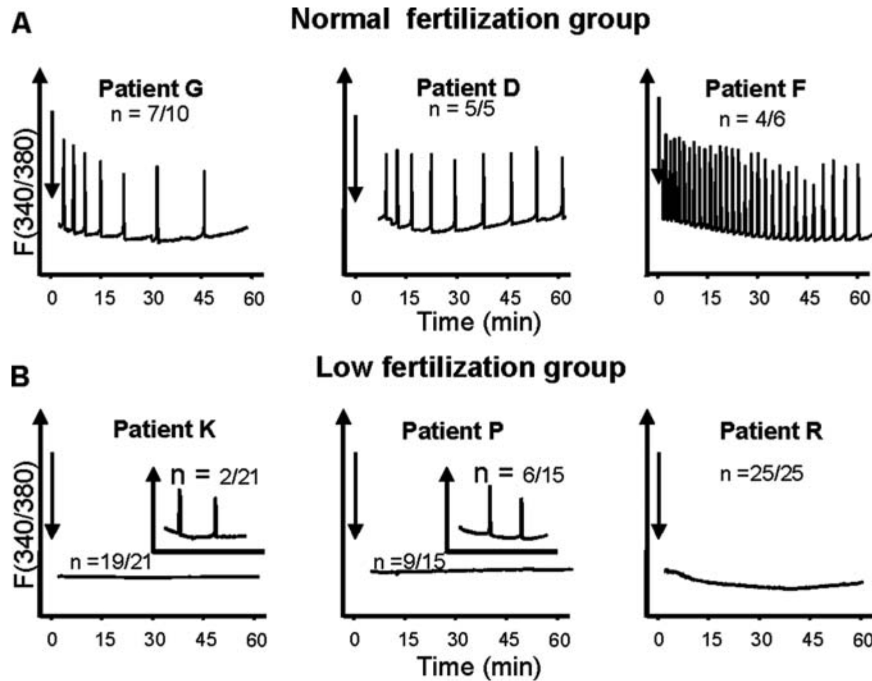

Figure 3 A comparison of calcium inducing ability between fertile and infertile sperm. Fertile human sperm induce calcium oscillations of characteristic amplitude and frequency when injected in mouse oocytes whereas infertile sperm do not. Downward arrows indicate the time of sperm injection. Reproduced from Yoon *et al*. (2008) with permission.

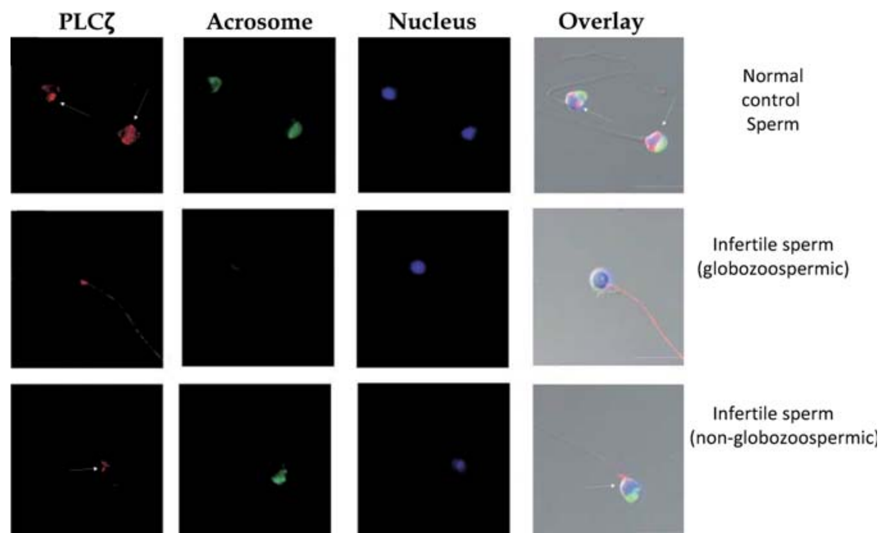

Figure 4 Localization of PLCζ in normal and infertile human sperm. PLCζ was detected in the equatorial region in the sperm from a healthy individual, was significantly diminished in the sperm from a globozoospermic patient, and was absent from a non-globozoospermic infertile patient. Reproduced from Heytens *et al*. (2009) with permission.

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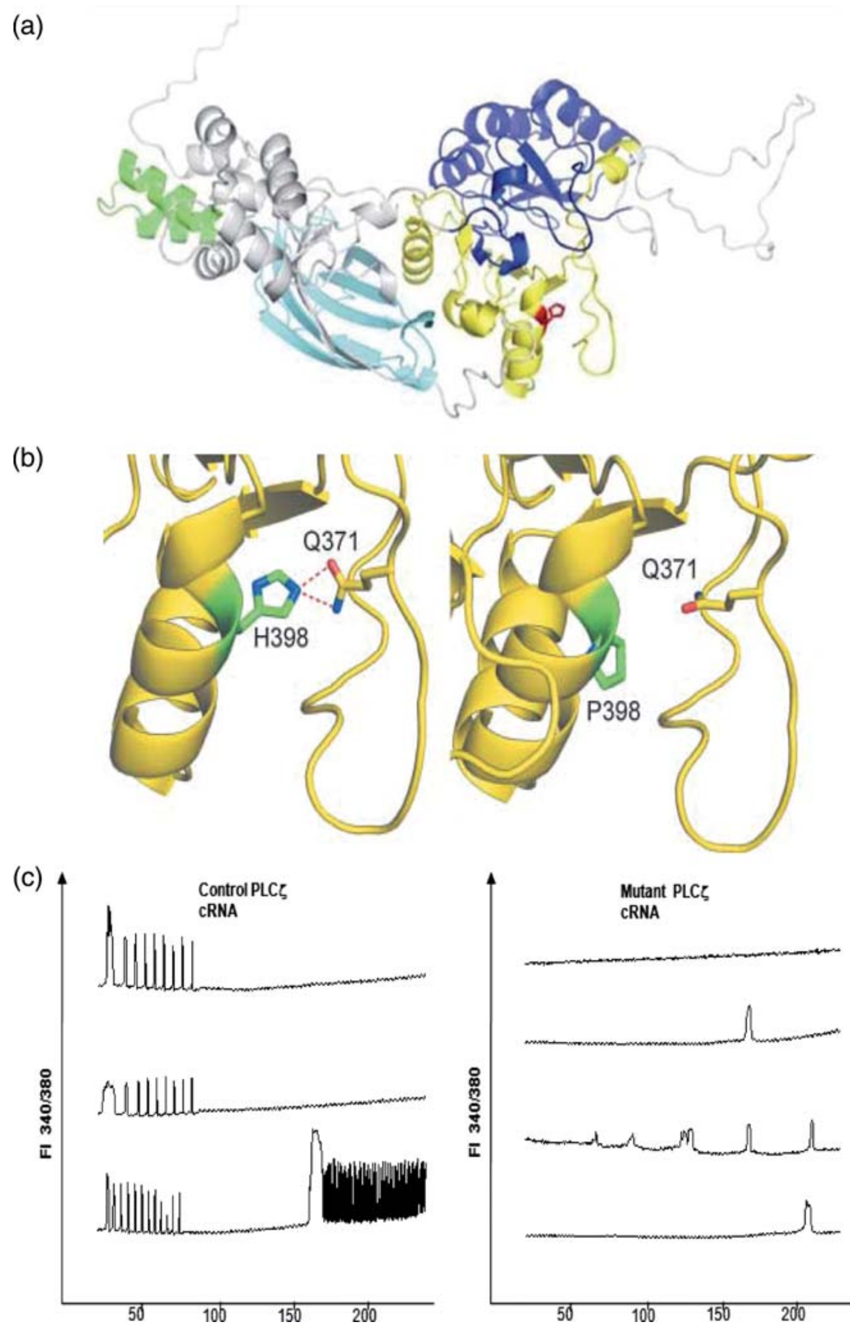

Figure 5 Histidine>Proline point mutation identified by Heytens *et al.* (2009) in an infertile male patient diagnosed with oocyte activation deficiency, (**a**) model of human PLCζ showing functional domains (green—EF-hand, blue—X catalytic domain, yellow—Y catalytic domain and cyan—C2 domain). Histidine398 is shown in red, (**b**) close-up of H398 showing side-chain–side-chain hydrogen bonds alongside a close-up of P398 in mutant PLCζ showing no side-chain–side-chain hydrogen bonds, (**c**) microinjection of wild type and mutant PLCζ into mouse oocytes and resulting calcium release patterns. Reproduced from Heytens *et al.* (2009) with permission.

cause of certain types of male factor infertility and oocyte activation failure. Recent studies have shown that the sperm of infertile men, which consistently fail IVF and ICSI are unable to produce Ca^{2+} oscillations upon injection into mouse oocytes, or produce oscillations which are uncharacteristic compared with those observed from fertile men, being reduced in both frequency and amplitude (Fig. 3; Yoon *et al.*, 2008; Heytens *et al.*, 2009). Furthermore, immunofluorescence and immunoblot analysis have revealed that infertile patients, whose sperm failed ICSI and were unable to produce Ca^{2+} oscillations, showed abnormalities in PLCζ expression (Fig. 4; Yoon *et al.*, 2008; Heytens *et al.*, 2009). Moreover, the activating ability of human sperm that have failed ICSI can be rescued upon co-injection with mouse PLCζ mRNA (Yoon *et al.*, 2008). Many of the

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patients in these studies were diagnosed with globozoospermia. Although analysis of the PLCζ gene revealed no major genetic abnormalities in globozoospermic men, the sperm of these patients exhibited reduced levels or absence of PLCζ expression (Yoon et al., 2008; Heytens et al., 2009). Interestingly, following the use of ICSI along with a Ca^{2+} ionophore, high rates of fertilization were observed, along with an ongoing pregnancy, when using globozoospermic sperm that had an absence of PLCζ expression (Taylor et al., 2010).

As discussed above, globozoospermia affects ~0.1% of infertile men (Dam et al., 2007) and is characterized by the presence of 100% round-headed sperm lacking an acrosome. As yet, it is unclear whether patients whose ejaculate contains both normal and globozoospermic cells (partial globozoospermia) suffer from a variation of the same syndrome (Dam et al., 2007). Yoon et al. (2008) reported absence of PLCζ in both the normal and round-headed sperm of a partial globozoospermic patient, in line with the inability of partial globozoospermic sperm cell types to activate mouse oocytes, as reported by Heindryckx et al. (2008). Numerous reports studying familial cases have suggested that globozoospermia is a genetic syndrome (Kullander and Rausing, 1975; Flörke-Gerloff et al., 1984; Dale et al., 1994; Kilani et al., 2004; Heindryckx et al., 2005). However, the specific mode of inheritance remains unclear (Dam et al., 2007), although recently it was reported that a mutation in the SPATA16 gene appears to be associated with certain types of globozoospermia in men (Dam et al., 2007).

Importantly, Heytens et al. (2009) identified a heterozygous substitution mutation in the coding sequence of PLCζ in a non-globozoospermic infertile male. This occurred within the Y domain at position 398 resulting in histidine (H398) being changed to proline (Heytens et al., 2009). Multiple sequence alignments confirmed the histidine to be conserved in this position across all mammalian PLCζs (Cox et al., 2002; Saunders et al., 2002; Yoneda et al., 2006; Young et al., 2009), chicken PLCζ (Coward et al., 2005), medaka (fish) PLCζ (Ito et al., 2008a, b), as well as all PLCδ isoforms (Ellis et al., 1998; Saunders et al., 2002), indicating that this residue may play an important role within the protein (Heytens et al., 2009). It is possible that the H398P mutation could cause important changes in PLCζ function as proline is a non-polar amino acid with a bulky planar ring side group, and a known disruptor of secondary structure and protein stability (Bajaj et al., 2007). Indeed, modelling studies of human PLCζ have predicted that the mutation could disrupt an alpha helix structure in the catalytic region and affect interactions with neighbouring amino acids (Fig. 5a and b; Heytens et al., 2009). In line with such predictions, injection of mutant human PLCζ cRNA into mouse oocytes resulted in highly abnormal Ca^{2+} transients, which were insufficient for oocyte activation, in contrast to oocytes injected with control wild type PLCζ which produced a series of Ca^{2+} oscillations characteristic of normal oocyte activation (Fig. 5c; Heytens et al., 2009).

Prospects for identifying further PLCζ mutants and potential for therapy and contraception

Although the findings of Yoon et al. (2008) and Heytens et al. (2009) provide the first clinical link between defects in PLCζ and human male infertility, this area of research is still very much in its infancy. Further analytical studies need to be undertaken to explore the precise effects of the H398P mutation upon PLCζ structure and function. To identify further patients with mutations in PLCζ, the incorporation of high-throughput genetic screening techniques, such as temperature gradient capillary electrophoresis, may be invaluable in increasing analytical efficiency. Yoon et al. (2008) and Heytens et al. (2009) focused on the PLCζ coding region, although both utilized PCR primers that also amplified at least 50 bp of the intronic sequences flanking each exon so as to include the intron/exon border in their analysis. Future studies could also screen introns and the PLCζ promoter region so as to include potential regulatory sites that might be mutated in some infertile patients.

For clinical purposes a combination of genetic analysis selectively targeting the PLCζ coding region, along with immunofluorescent and immunoblot analysis of sperm samples, may prove useful diagnostic tests for ART clinics. Currently, only heterologous ICSI models are available for testing the activation capacity of human sperm, such as MOAT (Rybouchkin et al., 1996; Heindryckx et al., 2008). Owing to ethical and legal restrictions, inconvenience and technical difficulty of mouse ICSI, it could be beneficial to develop simpler cell-free and enzymatic tests for PLCζ activity that might be used to diagnose infertility linked to oocyte activation deficiency. The identification of men with oocyte activation defects also raises the possibility of using recombinant PLCζ protein as a potential therapeutic agent.

The principle of using PLCζ therapeutically has already been demonstrated by Yoon et al. (2008), who 'rescued' the oocyte activating capability of globozoospermic sperm by the co-injection of PLCζ cRNA into mouse oocytes, although the pre- and post-implantation developmental potential could not be determined using this heterologous ICSI model. Rogers et al. (2004) also showed that PLCζ cRNA could activate aged human oocytes which had previously failed to fertilize. However, for clinical purposes, it would be necessary to use a recombinant protein form of PLCζ, for reasons already discussed.

Summary

Although assisted reproductive technologies have greatly reduced infertility, there are still cases of ICSI failure resulting from oocyte activation deficiency. Mammalian oocytes are activated by intracellular Ca^{2+} oscillations thought to be triggered by the sperm-specific phospholipase C, PLCζ. In this review, we have sought to evaluate the potential causes of oocyte activation failure and the potential role played by defective forms of PLCζ in causing infertility in men. Immunofluorescence and immunoblot analysis of PLCζ expression and localization in the sperm, combined with assays of PLCζ enzymatic activity, may have potential use as diagnostic tools. Although recently it has been shown that oocyte activation failure can be overcome clinically by artificial oocyte activators such as calcium ionophores, injection of recombinant PLCζ protein might be used in the future as a more physiological oocyte activation agent. This may be particularly important given recent studies showing that the precise pattern of Ca^{2+} oscillations at fertilization may influence both the efficiency and quality of embryo development.

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References

A long term analysis of the HFEA Register 1991–2006. July 2007, Online, Available at http://www.hfea.gov.uk/docs/Latest_long_term_data_analysis_report_91-06.pdf (22 May 2009, date last accessed).

Aarabi M, Qin Z, Xu W, Mewburn J, Oko R. Sperm borne protein, PAWP, initiates zygotic development in Xenopus laevis by eliciting intracellular calcium release. *Mol Reprod Dev* 2010;**77**:249–256.

Ajduk A, Ciemerych MA, Nixon V, Swann K, Maleszewski M. Fertilization differently affects the levels of cyclin B1 and M-phase promoting factor activity in maturing and metaphase II mouse oocytes. *Reproduction* 2008;**136**:741–752.

Alberio R, Zakhartchenko V, Motlik J, Wolf E. Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer. *Int J Dev Biol* 2001;**45**:797–809.

Ambasudhan R, Singh K, Agarwal JK, Singh SK, Khanna A, Sah RK, Singh I, Raman R. Idiopathic cases of male infertility from a region of India show low incidence of Y-chromosome microdeletion. *J Biosci* 2003; **28**:605–612.

Avenarius MR, Hildebrand MS, Zhang Y, Meyer NC, Smith LL, Kahrizi K, Najmabadi H, Smith RJ. Human male infertility caused by mutations in the CATSPER1 channel protein. *Am J Hum Genet* 2009;**84**:505–510.

Bajaj K, Madhusudhan MS, Adkar BV, Chakrabarti P, Ramakrishnan C, Sali A, Varadarajan R. Stereochemical criteria for prediction of the effects of proline mutations on protein stability. *PLoS Comput Biol* 2007;**3**:e241.

Baltaci V, Ayvaz OU, Unsal E, Aktaş Y, Baltacı A, Turhan F, Ozcan S, Sönmezer M. The effectiveness of intracytoplasmic sperm injection combined with piezoelectric stimulation in infertile couples with total fertilization failure. *Fertil Steril* 2009 (in press).

Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. *Fertil Steril* 1997;**68**:118–122.

Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. *Biochim Biophys Acta* 2009;**1793**:933–940.

Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod* 2007;**22**:1506–1512.

Bos-Mikich A, Whittingham D, Jones KT. Meiotic and mitotic Ca^{2+} oscillations affect cell composition in resulting blastocysts. *Dev Biol* 1997;**182**:172–179.

Bourne H, Liu DY, Clarke GN, Baker HW. Normal fertilization and embryo development by intracytoplasmic sperm injection of round-headed acrosomeless sperm. *Fertil Steril* 1995a;**63**:1329–1332.

Bourne H, Richings N, Harari O, Watkins W, Speirs AL, Johnston WI, Baker HW. The use of intracytoplasmic sperm injection for the treatment of severe and extreme male infertility. *Reprod Fertil Dev* 1995b;**7**:237–245.

Brevini TAL, Gandolfi F. Parthenotes as a source of embryonic stem cells. *Cell Prolif* 2008;**41**(Suppl. 1):20–30.

Carroll J. The initiation and regulation of Ca^{2+} signaling at fertilization in mammals. *Semin Cell Dev Biol* 2001;**12**:37–43.

Chemes HE, Brugo S, Zanchetti F, Carrere C, Lavieri JC. Dysplasia of the fibrous sheath: an ultrastructural defect of human spermatozoa associated with sperm immotility and primary sterility. *Fertil Steril* 1987;**48**:664–669.

Cheung A, Swann K, Carroll J. The ability to generate normal Ca^{2+} transients in response to spermatozoa develops during the final stages of oocyte growth and maturation. *Hum Reprod* 2000;**15**:1389–1395.

Cibelli J, Kiessling A, Cunniff K, Richards C, Lanza R, West M. Somatic cell nuclear transfer in humans: pronuclear and early embryonic development. *J Regen Med* 2001;**2**:25–31.

Cohen J, Stachecki J, Malter H, Wells D. Recent scientific developments in assisted reproduction. In: Brinsden PR (ed). *Textbook of In Vitro Fertilization and Assisted Reproduction*, 3rd edn. London, UK: Taylor & Francis, 2005.

Coward K, Ponting CP, Chang HY, Hibbitt O, Savolainen P, Jones KT, Parrington J. Phospholipase Czeta, the trigger of egg activation in mammals, is present in a non-mammalian species. *Reproduction* 2005; **130**:157–163.

Coward K, Kubota H, Parrington J. In vivo gene transfer in testis and sperm: developments and future applications. *Arch Androl* 2007; **53**:187–197.

Cox LJ, Larman MG, Saunders CM, Hashimoto K, Swann K, Lai FA. Sperm phospholipase C zeta from humans and cynomolgus monkeys triggers Ca^{2+} oscillations, activation and development of mouse oocytes. *Reproduction* 2002;**124**:611–623.

Cuthbertson KS, Cobbold PH. Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca^{2+}. *Nature* 1985; **316**:541–542.

Dale B, Iaccarino M, Fortunato A, Gragnaniello G, Kyozuka K, Tosti E. A morphological and functional study of fusibility in round-headed spermatozoa in the human. *Fertil Steril* 1994;**61**:336–340.

Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde RJ, Kremer JA. Globozoospermia revisited. *Hum Reprod Update* 2007;**13**:63–75.

de Fried EP, Ross P, Zang G, Divita A, Cunniff K, Denaday F, Salamone D, Kiessling A, Cibelli J. Human parthenogenetic blastocysts derived from noninseminated cryopreserved human oocytes. *Fertil Steril* 2008; **89**:943–947.

Deguchi R, Shirakawa H, Oda S, Mohri T, Miyazaki S. Spatiotemporal analysis of Ca^{2+} waves in relation to the sperm entry site and animal-vegetal axis during Ca^{2+} oscillations in fertilized mouse eggs. *Dev Biol* 2000;**218**:299–313.

Dirican EK, Isik A, Vicdan K, Sozen E, Suludere Z. Clinical pregnancies and livebirths achieved by intracytoplasmic injection of round headed acrosomeless spermatozoa with and without oocyte activation in familial globozoospermia: case report. *Asian J Androl* 2008;**10**:332–336.

Dohle GR, Jungwirth A, Kopa Z, Giwercman A, Diemer T, Hargreave TB. Guidelines on Male Infertility. European Association of Urology 2009. Available at http://www.uroweb.org/fileadmin/tx_eauguidelines/2007/Full/Male_Infertility.pdf . (22 May 2010, date last accessed).

Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S, Ozil JP. Egg-to-embryo transition is driven by differential responses to Ca(2+) oscillation number. *Dev Biol* 2002; **250**:280–291.

Ducibella T, Schultz RM, Ozil JP. Role of Calcium Signals in Early Development. *Semin Cel Dev Biol* 2006;**17**:324–332.

Dumollard R, Duchen M, Sardet C. Calcium signals and mitochondria at fertilization. Semin. *Cell Dev Biol* 2006;**17**:314–323.

Ebner T, Moser M, Sommergruber M, Jesacher K, Tews G. Complete oocyte activation failure after ICSI can be overcome by a modified injection technique. *Hum Reprod* 2004;**19**:1837–1841.

Elder K, Dale B. *In Vitro Fertilization*, 2nd edn. Cambridge, UK: Cambridge University Press, 2001.

Elliot DJ, Cooke HJ. The molecular genetics of male infertility. *Bioessays* 1997;**19**:801–809.

Ellis MV, James SR, Perisic O, Downes CP, Williams RL, Katan M. Catalytic domain of phosphoinositide-specific phospholipase C (PLC). Mutational

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analysis of residues within the active site and hydrophobic ridge of plcδ1. *J Biol Chem* 1998;**273**:11650–11659.

Eppig JJ, Schultz RM, O'Brien M, Chesnel F. Relationship between the developmental programs controlling nuclear and cytoplasmic maturation of mouse oocytes. *Dev Biol* 1994;**164**:1–9.

Evens EM. A global perspective on infertility: an under recognized public health issue. *Carolina Papers International Health* 2004;**18**:1–45.

Fissore RA, Pinto-Correira C, Robl JM. Inositol triphophateinduced Ca^{2+} release in the generation of calcium oscillations in bovine eggs. *Biol Reprod* 1995;**53**:766–774.

Fissore RA, Kurokawa M, Knott J, Zhang M, Smyth J. Mechanisms underlying oocyte activation and postovulatory ageing. *Reprod* 2002; **124**:745–754.

Flaherty SP, Payne D, Matthews CD. Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection. *Hum Reprod* 1998; **13**(Suppl. 1):155–164.

Flörke-Gerloff S, Töpfer-Petersen E, Müller-Esterl W, Mansouri A, Schatz R, Schirren C, Schill W, Engel W. Biochemical and genetic investigation of round-headed spermatozoa in infertile men including two brothers and their father. *Andrologia* 1984;**16**:187–202.

Forti G, Krausz C. Clinical review 100: Evaluation and treatment of the infertile couple. *J Clin Endocrinol Metab* 1998;**83**:4177–4188.

Fujimoto S, Yoshida N, Fukui T, Amanai M, Isobe T, Itagaki C, Izumi T, Perry AC. Mammalian phospholipase Czeta induces oocyte activation from the sperm perinuclear matrix. *Dev Biol* 2004;**274**: 370–383.

Fulton BP, Whittingham DG. Activation of mammalian oocytes by intracellular injection of calcium. *Nature* 1978;**273**:149–151.

Golan R, Weissenberg R, Oschry Y, Shocat L, Lewin LM. Spermatogenesis in the golden hamster during the first spermatogenic wave: a flow cytometric analysis. *Mol Reprod Dev* 2000;**55**:205–211.

Grasa P, Coward K, Young C, Parrington J. The pattern of localization of the putative oocyte activation factor, phospholipase C zeta, in uncapacitated, capacitated, and ionophore-treated human spermatozoa. *Hum Reprod* 2008;**23**:2513–2522.

Harada Y, Matsumoto T, Hirahara S, Nakashima A, Ueno S, Oda S, Miyazaki S, Iwao Y. Characterization of a sperm factor for egg activation at fertilization of the newt *Cynops pyrrhogaster*. *Dev Biol* 2007;**306**:797–808.

Heindryckx B, Van der Elst J, De Sutter P, Dhont M. Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. *Hum Reprod* 2005; **20**:2237–2241.

Heindryckx B, De Sutter P, Gerris J, Dhont M, Van der Elst J. Embryo development after successful somatic cell nuclear transfer to in vitro matured human germinal vesicle oocytes. *Hum Reprod* 2007; **22**:1982–1990.

Heindryckx B, De Gheselle S, Gerris J, Dhont M, De Sutter P. Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. *Reprod Biomed Online* 2008;**17**:662–668.

Heindryckx B, De Sutter P, Gerris J. Somatic nuclear transfer to in vitro-matured human germinal vesicle oocytes In: Simon C, Pellicer A (eds). *Stem Cells in Human Reproduction* 2009, 226–242.

Heytens E, Parrington J, Coward K, Young C, Lambrecht S, Yoon SY, Fissore RA, Hamer R, Deane CM, Ruas M *et al.* Reduced amounts and abnormal forms of phospholipase C zeta in spermatozoa from infertile men. *Hum Reprod* 2009;**24**:2417–2428.

Hicks SN, Jezyk MR, Gershburg S, Seifert JP, Harden TK, Sondek J. General and versatile autoinhibition of PLC isozymes. *Mol Cell* 2008; **31**:383–394.

Hull MG, Glazener CM, Kelly NJ, Conway DL, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, and outcome of infertility. *Br Med J* 1985; **291**:1693–1697.

Inhorn MC. Global infertility and the globalization of new reproductive technologies: illustrations from Egypt. *Soc Sci Med* 2003;**56**:1837–1851.

International Committee for Monitoring Assisted Reproductive Technology (ICMART):, de Mouzon J, Lancaster P, Nygren KG, Sullivan E, Zegers-Hochschild F, Mansour R, Ishihara O, Adamson D. World collaborative report on assisted reproductive technology, 2002. *Hum Reprod* 2009;**24**:2310–2320.

Ito M, Shikano T, Kuroda K, Miyazaki S. Relationship between nuclear sequestration of PLCzeta and termination of PLCzeta-induced Ca^{2+} oscillations in mouse eggs. *Cell Calcium* 2008a;**44**:400–410.

Ito M, Shikano T, Oda S, Horiguchi T, Tanimoto S, Awaji T, Mitani H, Miyazaki S. Difference in Ca2+ oscillation-inducing activity and nuclear translocation ability of PLCZ1, an egg-activating sperm factor candidate, between mouse, rat, human, and medaka fish. *Biol Reprod* 2008b;**78**:1081–1090.

Jones KT. Mammalian egg activation: from Ca^{2+} spiking to cell cycle progression. *Reproduction* 2005;**130**:813–823.

Jones KT. Intracellular calcium in the fertilization and development of mammalian eggs. *Clin Exp Pharmacol Physiol* 2007;**34**:1804–1089.

Jones KT, Soeller C, Cannell MB. The passage of Ca^{2+} and fluorescent markers between the sperm and egg after fusion in the mouse. *Development* 1998;**125**:4627–4635.

Jones KT, Matsuda M, Parrington J, Katan M, Swann K. Different Ca^{2+}-releasing abilities of sperm extracts compared with tissue extracts and phospholipase C isoforms in sea urchin egg homogenate and mouse eggs. *Biochem J* 2000;**346**:743–749.

Katan M. Families of phosphoinositide-specific phospholipase C: structure and function. *Biochim Biophys Acta*. 1998;**1436**:5–17.

Kilani ZM, Shaban MA, Ghunaim SD, Keilani SS, Dakkak AI. Triplet pregnancy and delivery after intracytoplasmic injection of round-headed spermatozoa. *Hum Reprod* 1998;**13**:2177–2179.

Kilani ZM, Ismail R, Ghunaim S, Mohamed H, Hughes D, Brewis I, Barrat CL. Evaluation and treatment of familial globozoospermia in five brothers. *Fertil Steril* 2004;**82**:1426–1429.

Kim ST, Cha YB, Park JM, Gye MC. Successful pregnancy and delivery from frozen-thawed embryos after intracytoplasmic sperm injection using round-headed spermatozoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome. *Fertil Steril* 2001;**75**:445–447.

Kimura Y, Yanagimachi R, Kuretake S, Bortkiewicz H, Perry AC, Yanagimachi H. Analysis of mouse oocyte activation suggests the involvement of sperm perinuclear material. *Biol Reprod* 1998; **58**:1407–1415.

Kishikawa H, Wakayama T, Yanagimachi R. Comparison of oocyte-activating agents for mouse cloning. *Cloning* 1999;**1**:153–159.

Kline D, Kline JT. Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Dev Biol* 1992; **149**:80–89.

Knott JG, Kurokawa M, Fissore RA, Schultz RM, Williams CJ. Transgenic RNA interference reveals role for mouse sperm phospholipase Czeta in triggering Ca^{2+} oscillations during fertilization. *Biol Reprod* 2005; **72**:992–996.

Kouchi Z, Fukami K, Shikano T, Oda S, Nakamura Y, Takenawa T, Miyazaki S. Recombinant phospholipase Czeta has high Ca^{2+} sensitivity and induces Ca^{2+} oscillations in mouse eggs. *J Biol Chem* 2004;**279**:10408–10412.

Kouchi Z, Shikano T, Nakamura Y, Shirakawa H, Fukami K, Miyazaki S. The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase Czeta. *J Biol Chem* 2005; **280**:21015–21021.

Downloaded from https://academic.oup.com/humupd/article/16/6/690/742219 by guest on 16 August 2022

Kullander S, Rausing A. On round-headed human spermatozoa. *Int J Fertil* 1975;**20**:33–40.

Kumtepe Y, Beyazyurek C, Cinar C, Ozbey I, Ozkan S, Cetinkaya K, Karlikaya G, Karagozoglu H, Kahraman S. A genetic survey of 1935 Turkish men with severe male factor infertility. *Reprod Biomed Online* 2009;**18**:465–474.

Kuroda K, Ito M, Shikano T, Awaji T, Yoda A, Takeuchi H, Kinoshita K, Miyazaki S. The role of X/Y linker region and N-terminal EF-hand domain in nuclear translocation and Ca^{2+} oscillation-inducing activities of phospholipase Czeta, a mammalian egg-activating factor. *J Biol Chem* 2006;**281**:27794–27805.

Kurokawa M, Sato K, Wu H, He C, Malcuit C, Black SJ, Fukami K, Fissore RA. Functional, biochemical, and chromatographic characterization of the complete [Ca2+]i oscillation-inducing activity of porcine sperm. *Dev Biol* 2005;**282**:376–392.

Kurokawa M, Yoon SY, Alfandari D, Fukami K, Sato K, Fissore RA. Proteolytic processing of phospholipase C zeta and $[Ca^{2+}]_i$ oscillations during mammalian fertilization. *Dev Biol* 2007;**312**: 407–418.

Kyono K, Kumagai S, Nishinaka C, Nakajo Y, Uto H, Toya M, Sugawara J, Araki Y. Birth and follow-up of babies born following ICSI using $SrCl_2$ oocyte activation. *Reprod Biomed Online* 2008; **17**:53–58.

Kyono K, Nakajo Y, Nishinaka C, Hattori H, Kyoya T, Ishikawa T, Abe H, Araki Y. A birth from the transfer of a single vitrified-warmed blastocyst using intracytoplasmic sperm injection with calcium ionophore oocyte activation in a globozoospermic patient. *Fertil Steril* 2009; **91**:931.e7–911.

Kyozuka K, Deguchi R, Mohri T, Miyazaki S. Injection of sperm extract mimics spatiotemporal dynamics of Ca2+ responses and progression of meiosis at fertilization of ascidian oocytes. *Development* 1998; **125**:4099–4105.

Lalancette C, Miller D, Li Y, Krawetz SA. Paternal contributions: new functional insights for spermatozoal RNA. *J Cell Biochem* 2008; **104**:1570–1579.

Larman MG, Saunders CM, Carroll J, Lai FA, Swann K. Cell cycle-dependent Ca^{2+} oscillations in mouse embryos are regulated by nuclear targeting of PLCzeta. *J Cell Sci* 2004;**117**:2513–2521.

Ledger WL. Demographics of Infertility. *Reprod Biomed Online* 2008; **18**(Suppl. 2):11–14.

Lee SB, Varnai P, Balla A, Jalink K, Rhee SG, Balla T. The pleckstrin homology domain of phosphoinositide-specific phospholipase C delta (4) is not a critical determinant of the membrane localization of the enzyme. *J Biol Chem* 2004;**279**:24362–24371.

Lin H, Lei J, Wininger D, Nguyen MT, Khanna R, Hartmann C, Yan WL, Huang SC. Multilineage potential of homozygous stem cells derived from metaphase II oocytes. *Stem Cells* 2003;**21**:152–161.

Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steirteghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. *Hum Reprod* 1995;**10**:626–629.

Machaty Z. Activation of oocytes after nuclear transfer. *Methods Mol Biol* 2006;**348**:43–58.

Maheshwari A, Hamilton M, Bhattacharya S. Effect of female age on the diagnostic categories of infertility. *Hum Reprod* 2008;**23**:538–542.

Mahutte NG, Arici A. Failed Fertilization: is it possible? *Curr Opin Obstet Gynecol* 2003;**15**:211–218.

Mai Q, Yu Y, Li T, Wang L, Chen MJ, Huang SZ, Zhou C, Zhou Q. Derivation of human embryonic stem cell lines from parthenogenetic blastocysts. *Cell Res* 2007;**17**:1008–1019.

Mangoli V, Dandekar S, Desai S, Mangoli R. The outcome of ART in males with impaired spermatogenesis. *J Hum Reprod Sci* 2008;**1**:73–76.

Marangos P, FitzHarris G, Carroll J. Ca^{2+} oscillations at fertilization in mammals are regulated by the formation of pronuclei. *Development* 2003;**130**:1461–1472.

Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. *Nat Med* 2008;**14**:1197–1213.

McVeigh E, Homburg R, Guillebaud J. *Oxford Handbook of Reproductive Medicine and Family Planning*, 1st edn. Oxford, UK: Oxford University Press, 2008.

Meacham RB, Joyce GF, Wise M, Kparker A, Niederberger C. Urologic diseases in America project. Male infertility. *J Urol* 2007; **177**:2058–2066.

Miyazaki S, Ito M. Calcium signals for egg activation in mammals. *J Pharmacol Sci* 2006;**100**:545–552.

Miyazaki S, Shirakawa H, Nakada K, Honda Y. Essential role of the inositol 1,4,5-trisphosphate receptor/Ca2+ release channel in Ca^{2+} waves and Ca^{2+} oscillations at fertilization of mammalian eggs. *Dev Biol* 1993; **158**:62–78.

Mizushima S, Takagi S, Ono T, Atsumi Y, Tsukada A, Saito N, Shimada K. Phospholipase Czeta mRNA expression and its potency during spermatogenesis for activation of quail oocyte as a sperm factor. *Mol Reprod Dev* 2009;**76**:1200–1207.

Nagafuchi S, Namiki M, Nakahori Y, Okuyama A, Nakagome Y. A minute deletion of the Y chromosome in men with azoospermia. *J Urol* 1993; **150**:1155–1157.

Nakamura S, Terada Y, Horiuchi T, Emuta C, Murakami T, Yaegashi N, Okamura K. Human sperm aster formation and pronuclear decondensation in bovine eggs following intracytoplasmic sperm injection using a Piezo-driven pipette: a novel assay for human sperm centrosomal function. *Biol Reprod* 2001;**65**:1359–1363.

Nakamura S, Terada Y, Horiuchi T, Emuta C, Murakami T, Yaegashi N, Okamura K. Analysis of the human sperm centrosomal function and the oocyte activation ability in a case of globozoospermia, by ICSI into bovine oocytes. *Hum Reprod* 2002;**17**:2930–2934.

Nakanishi T, Ishibashi N, Kubota H, Inoue K, Ogonuki N, Ogura A, Kashiwabara S, Baba T. Birth of normal offspring from mouse eggs activated by a phospholipase C zeta protein lacking three EF-hand domains. *J Reprod Dev* 2008;**54**:244–249.

Nasr-Esfahani MH, Deemeh MR, Tavalaee M. Artificial oocyte activation and intracytoplasmic sperm injection. *Fertil Steril* Advance Access published May 21, 2009.

National Collaborating Centre for Women's and Children's Health. Fertility: assessment and treatment for people with fertility problems. Available at www.rcog.org.uk/files/rcog-corp/uploaded-files/NEBFertilityFull.pdf (8 January 2010, date last accessed).

Nomikos M, Blayney LM, Larman MG, Campbell K, Rossbach A, Saunders CM, Swann K, Lai FA. Role of phospholipase C-zeta domains in Ca^{2+}-dependent phosphatidylinositol 4,5-bisphosphate hydrolysis and cytoplasmic Ca^{2+} oscillations. *J Biol Chem* 2005; **280**:31011–31018.

Nomikos M, Mulgrew-Nesbitt A, Pallavi P, Mihalyne G, Zaitseva I, Swann K, Lai FA, Murray D, McLaughlin S. Binding of phosphoinositide-specific phospholipase C-zeta (PLC-zeta) to phospholipid membranes: potential role of an unstructured cluster of basic residues. *J Biol Chem* 2007;**282**:16644–16653.

Ombelet W, Cooke I, Dyer S, Serour G, Devroey P. Infertility and the provision of infertility medical services in developing countries. *Hum Reprod Update* 2008;**14**:605–621.

Ozil JP, Huneau D. Activation of rabbit oocytes: the impact of the Ca^{2+} signal regime on development. *Development* 2001;**128**: 917–928.

Ozil JP, Markoulaki S, Toth S, Matson S, Banrezes B, Knott JG, Schultz RM, Huneau D, Ducibella T. Egg activation events are regulated by the

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duration of a sustained [Ca2+]cyt signal in the mouse. *Dev Biol* 2005; **282**:39–54.

Ozil JP, Banrezes B, Toth S, Pan H, Schultz RM. Ca2+ oscillatory pattern in fertilized mouse eggs affects gene expression and development to term. *Dev Biol* 2006;**300**:534–544.

Paffoni A, Brevini TA, Somigliana E, Restelli L, Gandolfi F, Ragni G. In vitro development of human oocytes after parthenogenetic activation or intracytoplasmic sperm injection. *Fertil Steril* 2007; **87**:77–82.

Parrington J. Does a soluble sperm factor trigger calcium release in the egg at fertilization? *J Androl* 2001;**22**:1–11.

Parrington J, Jones KT, Lai A, Swann K. The soluble sperm factor that causes Ca^{2+} release from sea-urchin (Lytechinus pictus) egg homogenates also triggers Ca^{2+} oscillations after injection into mouse eggs. *Biochem J* 1999;**341**:1–4.

Parrington J, Lai FA, Swann K. The soluble mammalian sperm factor protein that triggers Ca2+ oscillations in eggs: evidence for expression of mRNA(s) coding for sperm factor protein(s) in spermatogenic cells. *Biol Cell* 2000;**92**:267–275.

Parrington J, Jones ML, Tunwell R, Devader C, Katan M, Swann K. Phospholipase C isoforms in mammalian spermatozoa: potential components of the sperm factor that causes Ca^{2+} release in eggs. *Reproduction* 2002;**123**:31–39.

Parrington J, Davis LC, Galione A, Wessel G. Flipping the switch: how a sperm activates the egg at fertilization. *Dev Dyn* 2007;**236**: 2027–2038.

Perry AC, Wakayama T, Yanagimachi R. A novel trans-complementation assay suggests full mammalian oocyte activation is coordinately initiated by multiple, submembrane sperm components. *Biol Reprod* 1999;**60**:747–755.

Platts AE, Dix DJ, Chemes HE, Thompson KE, Goodrich R, Rockett JC, Rawe VY, Quintana S, Diamond MP, Strader LF *et al.* Success and failure in human spermatogenesis as revealed by teratozoospermic RNAs. *Hum Mol Genet* 2007;**16**:763–773.

Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. *Singapore Med J* 2009;**50**:336–347.

Publicover S, Harper CV, Barratt C. $[Ca^{2+}]_i$ Signalling in Sperm – Making the Most of What You've Got. *Nat Cell Biol* 2007;**9**:235–242.

Rawe VY, Terada Y, Nakamura S, Chillik C, Brugo Olmedo S, Chemes HE. A pathology of the sperm centriole responsible for defective sperm aster formation, syngamy and cleavage. *Hum Reprod* 2002; **17**:2344–2349.

Rawe VY, Díaz ES, Abdelmassih R, Wójcik C, Morales P, Sutovsky P, Chemes HE. The role of sperm proteasomes during sperm aster formation and early zygote development: implications for fertilization failure in humans. *Hum Reprod* 2008;**23**:573–580.

Rebecchi MJ, Pentyala SN. Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol Rev* 2000; **80**:1291–1335.

Revazova ES, Turovets NA, Kochetkova OD, Kindarova LB, Kuzmichev LN, Janus JD, Pryzhkova MV. Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* 2007;**9**:432–449.

Rhee SG. Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem* 2001;**70**:281–312.

Rice A, Parrington J, Jones KT, Swann K. Mammalian sperm contain a Ca(2+)-sensitive phospholipase C activity that can generate InsP(3) from PIP(2) associated with intracellular organelles. *Dev Biol* 2000; **228**:125–135.

Rogers NT, Hobson E, Pickering S, Lai FA, Braude P, Swann K. Phospholipase Czeta causes Ca^{2+} oscillations and parthenogenetic activation of human oocytes. *Reproduction* 2004;**128**:697–702.

Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, Dorin J, Cooke HJ. The mouse DAZLA gene encodes a cytoplasmic protein essential for spermatogenesis. *Nature* 1997;**389**:73–77.

Rybouchkin A, Dozortsev D, Pelinck MJ, De Sutter P, Dhont M. Analysis of the oocyte activation capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes. *Hum Reprod* 1996;**11**:2170–2175.

Rybouchkin AV, Van der Straeten F, Quatacker J, De Sutter P, Dhont M. Fertilization and pregnancy after assisted oocyte activation and intracytoplasmic sperm injection in a case of round-headed sperm associated with deficient oocyte activation capacity. *Fertil Steril* 1997; **68**:1144–1147.

Sasaki S, Kojima Y, Kubota H, Tatsura H, Hayashi Y, Kohri K. Effects of the gene transfer into sperm mediated by liposomes on sperm motility and fertilization in vitro. *Hinyokika Kiyo* 2000;**46**:591–595.

Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K, Lai FA. PLC zeta: a sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development. *Development* 2002; **129**:3533–3544.

Saunders CM, Swann K, Lai FA. PLC zeta, a sperm-specific PLC and its potential role in fertilization. *Biochem Soc Symp* 2007;**74**:23–36.

Sone Y, Ito M, Shirakawa H, Shikano T, Takeuchi H, Kinoshita K, Miyazaki S. Nuclear translocation of phospholipase C-zeta, an egg-activating factor, during early embryonic development. *Biochem Biophys Res Commun* 2005;**330**:690–694.

Sousa M, Tesarik J. Ultrastructural analysis of fertilization failure after intracytoplasmic sperm injection. *Hum Reprod* 1994;**9**:2374–2380.

Spadafora C. Endogenous reverse transcriptase: a mediator of cell proliferation and differentiation. *Cytogenet Genome Res* 2004;**105**: 346–350.

Sutovsky P, Manandhar G, Wu A, Oko R. Interactions of sperm perinuclear theca with the oocyte: implications for oocyte activation, anti-polyspermy defense, and assisted reproduction. *Microsc Res Tech* 2003;**61**:362–378.

Swain JE, Pool TB. ART failure: oocyte contributions to unsuccessful fertilization. *Hum Reprod Update* 2008;**14**:431–446.

Swann K, Ozil JP. Dynamics of the calcium signal that triggers mammalian egg activation. *Int Rev Cytol* 1994;**152**:183–222.

Swann K, Larman MG, Saunders CM, Lai FA. The cytosolic sperm factor that triggers Ca^{2+} oscillations and egg activation in mammals is a novel phospholipase C: PLCzeta. *Reproduction* 2004;**127**:431–439.

Swann K, Saunders CM, Rogers NT, Lai FA. PLCzeta (zeta): a sperm protein that triggers Ca^{2+} oscillations and egg activation in mammals. *Semin Cell Dev Biol* 2006;**17**:264–273.

Swann K, Yu Y. The dynamics of calcium oscillations that activate mammalian eggs. *Int J Dev Biol* 2008;**52**:585–594.

Taylor SL, Yoon SY, Morshedi MS, Lacey DR, Jellerette T, Fissore RA, Oehninger S. Complete globozoospermia associated with PLCzeta deficiency treated with calcium ionophore and ICSI results in pregnancy. *Reprod Biomed Online* 2010;**20**:559–564.

Tejara A, Mollá M, Muriel L, Remohi J, Pellicer A, De Pablo JL. Successful pregnancy and childbirth after intracytoplasmic sperm injection with calcium ionophore oocyte activation in a globozoospermic patient. *Fertil Steril* 2008;**90**:1202.e1–1205.

Terada Y, Hasegawa H, Takahashi A, Ugajin T, Yaegashi N, Okamura K. Successful pregnancy after oocyte activation by a calcium ionophore for a patient with recurrent intracytoplasmic sperm injection failure, with an assessment of oocyte activation and sperm centrosomal function using bovine eggs. *Fertil Steril* 2009;**91**:935.e11–934.

Tesarik J, Rienzi L, Ubaldi F, Mendoza C, Greco E. Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne oocyte activation failures. *Fertil Steril* 2002;**78**:619–624.

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The ESHRE Capri Workshop Group. Intracytoplasmic sperm injection (ICSI) in 2006: evidence and evolution. *Hum Reprod Update* 2007; **13**:515–526.

Tóth S, Huneau D, Banrezes B, Ozil JP. Egg activation is the result of calcium signal summation in the mouse. *Reproduction* 2006;**131**:27–34.

Trokoudes KM, Danos N, Kalogirou L, Vlachou R, Lysiotis T, Georghiades N, Lerios S, Kyriacou K. Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment. *Hum Reprod* 1995;**10**:880–882.

Turner RM, Musse MP, Mandal A, Klotz K, Jayes FC, Herr JC, Gerton GL, Moss SB, Chemes HE. Molecular genetic analysis of two human sperm fibrous sheath proteins, AKAP4 and AKAP3, in men with dysplasia of the fibrous sheath. *J Androl* 2001;**22**:302–315.

Van Blerkom J. Sperm centrosome dysfunction: a possible new class of male factor infertility in the human. *Mol Hum Reprod* 1996;**2**:349–354.

Van Blerkom J, Davis P, Mathwig V, Alexander S. Domains of high-polarized and low-polarized mitochondria may occur in mouse and human oocytes and early embryos. *Hum Reprod* 2002;**17**:393–406.

Vogt PH, Edelmann A, Hirschmann P, Köhler MR. The azoospermia factor (AZF) of the human Y chromosome in Yq11: function and analysis in spermatogenesis. *Reprod Fertil Dev* 1995;**7**:685–693.

Whitaker M. Calcium at fertilization and in early development. *Physiol Rev* 2006;**86**:25–88.

Wilkes S, Chinn DJ, Murdoch A, Rubin G. Epidemiology and management of infertility: a population-based study in UK primary care. *Fam Pract* 2009;**26**:269–274.

Wu AT, Sutovsky P, Manandhar G, Xu W, Katayama M, Day BN, Park KW, Yi YJ, Xi YW, Prather RS *et al*. PAWP, a sperm-specific WW domain-binding protein, promotes meiotic resumption and pronuclear development during fertilization. *J Biol Chem* 2007; **282**:12164–12175.

Yanagida K. Complete fertilization failure in ICSI. *Hum Cell* 2004;**17**: 187–193.

Yanagida K, Katayose H, Yazawa H, Kimura Y, Sato A, Yanagimachi H, Yanagimachi R. Successful fertilization and pregnancy following ICSI and electrical oocyte activation. *Hum Reprod* 1999;**14**:1307–1311.

Yanagida K, Morozumi K, Katayose H, Hayashi S, Sato A. Successful pregnancy after ICSI with strontium oocyte activation in low rates of fertilization. *Reprod Biomed Online* 2006;**13**:801–806.

Yanagida K, Fujikura Y, Katayose H. The present status of artificial oocyte activation in assisted reproductive technology. *Reprod Med Biol* 2008; **7**:133–142.

Yoda A, Oda S, Shikano T, Kouchi Z, Awaji T, Shirakawa H, Kinoshita A, Miyazaki S. Ca^{2+} oscillation-inducing phospholipase C zeta expressed in mouse eggs is accumulated to the pronucleus during egg activation. *Dev Biol* 2004;**268**:245–257.

Yoneda A, Kashima M, Yoshida S, Terada K, Nakagawa S, Sakamoto A, Hayakawa K, Suzuki K, Ueda J, Watanabe T. Molecular cloning, testicular postnatal expression, and oocyte-activating potential of porcine phospholipase C zeta. *Reproduction* 2006;**132**: 393–401.

Yoon SY, Fissore RA. Release of phospholipase C zeta and $[Ca^{2+}]_i$ oscillation-inducing activity during mammalian fertilization. *Reproduction* 2007;**134**:695–704.

Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, Coward K, Parrington J, Grow D, Cibelli JB, Visconti PE *et al*. Human sperm devoid of PLC, zeta 1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. *J Clin Invest* 2008; **118**:3671–3681.

Young C, Grasa P, Coward K, Davis LC, Parrington J. Phospholipase C zeta undergoes dynamic changes in its pattern of localization in sperm during capacitation and the acrosome reaction. *Fertil Steril* 2009; **91**:2230–2242.

Yu Y, Saunders CM, Lai FA, Swann K. Preimplantation development of mouse oocytes activated by different levels of human phospholipase C zeta. *Hum Reprod* 2008;**23**:365–373.

Yu Y, Mai Q, Chen X, Wang L, Gao L, Zhou C, Zhou Q. Assessment of the developmental competence of human somatic cell nuclear transfer embryos by oocyte morphology classification. *Hum Reprod* 2009; **24**:649–657.

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