Zygote

cambridge.org/zyg

Exonic genetic variants associated with unexpected fertilization failure and zygotic arrest after ICSI: a systematic review

Marc Torra-Massana, Amelia Rodríguez and Rita Vassena*

Eugin, Balmes 236, 08006, Barcelona, Spain

Review Article

Cite this article: Torra-Massana M *et al*. (2023) Exonic genetic variants associated with unexpected fertilization failure and zygotic arrest after ICSI: a systematic review. *Zygote*. **31**: 316–341. doi: 10.1017/S096719942300014X

Received: 22 June 2022
Revised: 20 December 2022
Accepted: 7 March 2023
First published online: 22 May 2023

Keywords:
Fertilization failure; Gene sequencing; ICSI; Infertility; Oocyte activation failure; Variants; Zygotic arrest

Corresponding author: Marc Torra-Massana, Eugin, Balmes 236, 08006, Barcelona, Spain. E-mail: mtorra@eugin.es

*Current address: Fecundis, Barcelona, Spain.

Summary

Fertilization failure (FF) and zygotic arrest after ICSI have a huge effect on both patients and clinicians, but both problems are usually unexpected and cannot be properly diagnosed. Fortunately, in recent years, gene sequencing has allowed the identification of multiple genetic variants underlying failed ICSI outcomes, but the use of this approach is still far from routine in the fertility clinic. In this systematic review, the genetic variants associated with FF, abnormal fertilization and/or zygotic arrest after ICSI are compiled and analyzed. Forty-seven studies were included. Data from 141 patients carrying 121 genetic variants affecting 16 genes were recorded and analyzed. In total, 27 variants in *PLCZ1* (in 50 men) and 26 variants in *WEE2* (in 24 women) are two of the factors related to oocyte activation failure that could explain a high percentage of male-related and female-related FF. Additional variants identified were reported in *WBP2NL*, *ACTL9, ACTLA7,* and *DNAH17* (in men), and *TUBB8*, *PATL2*, *TLE6*, *PADI6*, *TRIP13*, *BGT4, NLRP5, NLRP7*, *CDC20* and *ZAR1* (in women). Most of these variants are pathogenic or potentially pathogenic (89/121, 72.9%), as demonstrated by experimental and/or *in silico* approaches. Most individuals carried bi-allelic variants (89/141, 63.1%), but pathogenic variants in heterozygosity have been identified for *PLCZ1* and *TUBB8*. Clinical treatment options for affected individuals, such as chemical-assisted oocyte activation (AOA) or *PLCZ1* cRNA injection in the oocyte, are still experimental. In conclusion, a genetic study of known pathogenic variants may help in diagnosing recurrent FF and zygotic arrest and guide patient counselling and future research perspectives.

Introduction

Intracytoplasmic sperm injection (ICSI) (Palermo *et al*., 1992) has revolutionized the treatment of male factor infertility. Most studies have reported that ICSI with ejaculated spermatozoa results in a mean fertilization rate (FR) of 70–75% (Flaherty *et al*., 1998; Pujol *et al*., 2018). However, total fertilization failure (FF) after ICSI, i.e. the absence of normally fertilized zygotes (containing two polar bodies and two pronuclei) at 16–19 h post-insemination, is a persistent problem in fertility clinics. In many cases, FF is associated with a low oocyte yield: FF occurs in ~17% of cycles with fewer than three oocytes, and in ~31% of cycles when only one mature oocyte is available (Flaherty *et al*., 1998; Hojnik and Kovačič, 2019). However, its incidence remains at 1–3% in all cycles even when inseminating a high number of oocytes (Flaherty *et al*., 1998; Palermo *et al*., 2017). FF can be repetitive, and it can happen even when morphologically normal sperm and oocytes are used for ICSI.

The most common aetiology of FF is by far oocyte activation failure (OAF; the failure to elicit the release from the meiotic block in response to sperm entry in the oocyte), accounting for ~40–80% of non-fertilized oocytes and characterized by the absence of pronuclei (Sousa and Tesarik, 1994; Flaherty *et al*., 1998; Rawe *et al*., 2000). Abnormal fertilization is also a common problem, characterized by the presence of an abnormal number of pronuclei (1PN, 3PN, or ≥3PN), representing ~10% of all inseminated oocytes (Balakier, 1993). In some cases, oocyte activation occurs normally and 2PN zygotes are formed, but the embryo fails to undergo the first mitotic division and arrests its development at the pronuclear stage, something commonly referred to as zygote arrest (Zamora *et al*., 2011).

Independently of their origin, all these situations result in a lack of viable embryos before 24 h post-insemination. Often, FF and zygotic arrest are unexplained and unexpected, and cannot be easily resolved using routine *in vitro* fertilization (IVF) methods. They invariably imply a high economic and psychological effect for the patients and are associated with difficult clinical management and counselling. Currently, treatment options for these patients are limited to the use of donor gametes, assisted oocyte activation (AOA) in some cases, or trying a new cycle using the same gametes. Nevertheless, this last option is usually accompanied by low expectations of success.

Some of the cellular alterations underlying FF are oocyte cytoplasmic immaturity (Balakier *et al.*, 2004), lack of sperm nucleus decondensation and/or aster formation (Rawe *et al.*, 2008), defects in oocyte spindle and cytoskeletal function (Rawe *et al.*, 2000), the sperm's inability to trigger calcium oscillations (Yoon *et al.*, 2008), or the inability of the oocyte machinery to respond to its stimuli (Yeste *et al.*, 2016; Ferrer-Buitrago *et al.*, 2019). Similarly, zygote arrest seems to be associated with specific morphological features in the zygote, such as uneven pronuclear size or reduced organization of nucleolar precursor bodies (Sadowy *et al.*, 1998; Zamora *et al.*, 2011), and chromosomal abnormalities (Benkhalifa *et al.*, 2003). Several proteins participate in oocyte activation and early fertilization events, including sperm PLCζ, as well as oocyte IP_3 receptors, kinases such as CAMKII, PKC, WEE2, MLCK, MOS and MAPK, calcium channels such as TRPM7 and CaV3.2, factors involved in sperm decondensation such as NPM2, HIRA, and the recently discovered SRPK1, and proteins of the subcortical maternal complex (SCMC), among many others (Moos *et al.*, 1995; Ducibella and Fissore, 2008; Oh *et al.*, 2011; Yeste *et al.*, 2016; Bernhardt *et al.*, 2017; Gou *et al.*, 2020). However, a causal link between genetic alterations in these factors and FF has remained largely speculative.

Fortunately, during the last decade, multiple genetic variants have been associated with FF or zygote arrest after ICSI. The objective of this review was to compile all the genetic variants identified in infertile patients experiencing FF, abnormal fertilization, and zygotic arrest after ICSI, and comprehensively analyze them in terms of pathogenesis, inheritance, frequency, molecular effects on protein function and fertilization and treatment options. Our objective is to provide a tool for the diagnosis and counselling of infertile patients.

Materials and methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati *et al.*, 2009).

Search strategy

A systematic literature search was performed using the PubMed database to identify research articles reporting genetic variants affecting genes with a demonstrated or suspected role during fertilization through ICSI (from insemination until first mitotic division), identified in infertile patients. The results were restricted to studies published in the English language from 2006 to 2021. Some factors participate in a wide range of cellular processes (from oocyte maturation to first embryonic cellular divisions) and their alterations can result in diverse clinical consequences. For this reason two terms associated with phenotypes downstream and upstream from the zygotic stage were included in the systematic search: oocyte maturation arrest and embryonic arrest. In total, seven terms were considered to identify the infertility phenotypes compatible with the objective of the present review, and key words were combined in the following search equation:

((((fertilization failure [Title/Abstract] OR oocyte activation [Title/Abstract] OR zygotic arrest [Title/Abstract] OR cleavage failure [Title/Abstract] OR abnormal fertilization [Title/Abstract] OR oocyte maturation arrest [Title/Abstract] OR embryonic arrest [Title/Abstract]) AND (mutation [Title/Abstract] OR sequencing [Title/Abstract] OR variant [Title/Abstract] OR variation [Title/Abstract]) AND (ICSI [Title/Abstract] OR *in vitro* fertilization [Title/Abstract] OR infertility [Title/Abstract] OR patient [Title/Abstract]))) AND English [Language]).

The reports matching the search question were retrieved, and their reference lists were manually reviewed to identify additional studies. In addition, using the same terms, additional studies were manually identified from peer-reviewed abstracts presented in ESHRE Annual Meetings from 2014 to 2021.

Study selection

Studies were screened for eligibility by titles and abstracts. Importantly, we only considered genetic variants identified in patients presenting gametes without severe morphological alterations (i.e. globozoospermia, oocytes with giant polar bodies, etc.), therefore the cases in which the FF or zygotic arrest problem are usually unexpected. All studies including infertile men or women undergoing ICSI in which sequencing was used to identify genetic variants in genes associated with fertilization were included. The studies that (1) did not use gene sequencing to report genetic variants, (2) exclusively included patients with phenotypes not matching the research question, or (3) used other insemination techniques (such as conventional IVF), were discarded. Study selection and data extraction (i.e. building the lists of genetic variants and subsequent analysis) were performed and verified by two independent investigators.

Study outcomes, data extraction and analysis

Data concerning infertility phenotype/s, experimental or *in silico* validation of the variant effect on fertilization, affected protein domains, inheritance, zygosity, treatment options, and genetic counselling were manually extracted from tables and/or text, collected and analyzed. To identify clear cases of FF that are likely to have been caused by alterations in the gametes, the inclusion criteria for patients in the studies included in this review were: four or more mature oocytes [morphologically normal and recognizable as PB1/metaphase II (MII) oocytes] inseminated by ICSI, and total FF or low FR (≤25% 2PN zygotes) and/or ≤25% cleavage rate among fertilized oocytes (2PN).

To analyze the population frequency of the identified variants, the following public databases were considered: GnomAD_exome (https://gnomad.broadinstitute.org/) and TOPMED.

Results

Results of a systematic search of the literature

The initial systematic search of the PubMed database identified 108 studies. After screening of titles and abstracts, 67 studies were selected for detailed analysis of the full-text manuscript, 40 of which met the inclusion criteria. Analysis of the bibliographical references in each manuscript allowed for the inclusion of four additional studies, and three studies were identified from ESHRE Annual Meeting (2014–2020). In total, 47 studies were finally included in the present review (Figure 1).

The 47 studies reported 121 exonic variants in 141 infertile patients. Specifically, 38 variants were identified in 61 men, while 83 variants were identified in 80 women. These variants affected 16 genes: *PLCZ1*, *WBP2NL*, *ACTL9*, *ACTLA7* and *DNAH17* in infertile men, and *WEE2*, *TUBB8*, *PATL2*, *TLE6*, *PADI6*, *TRIP13*, *BGT4, NLRP5, NLRP7*, *CDC20* and *ZAR1* in infertile women. Among the 121 variants reported, 58 (47.9%) were not present in the public databases analyzed and, except for some cases, the

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

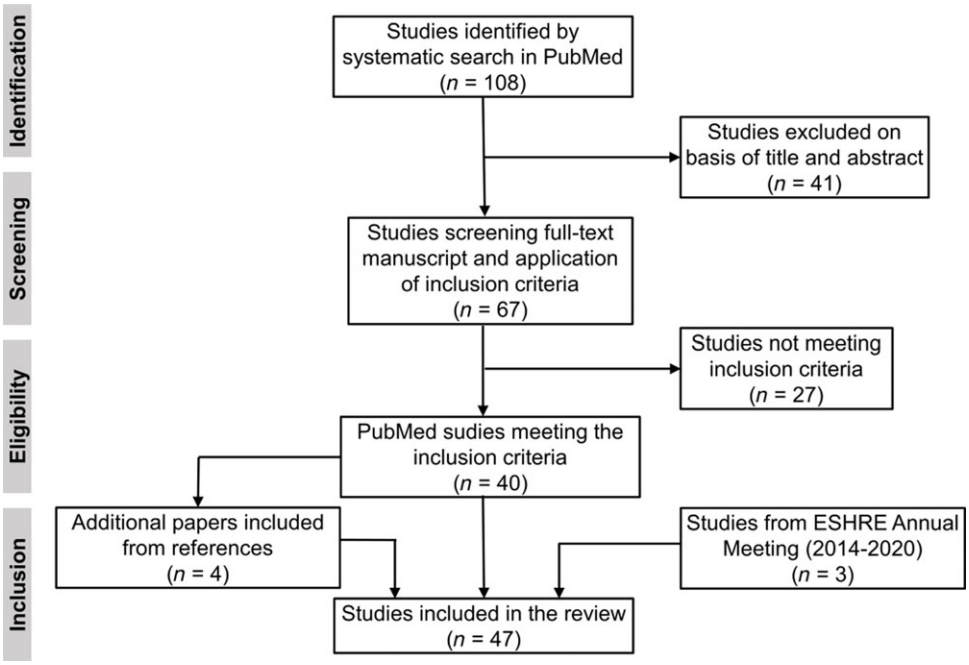

Figure 1. PRISMA flow diagram of identification, screening, eligibility, and inclusion steps.

Minor Allele Frequency (MAF) reported for most of the genetic variants present in the dbSNP database was very low (Table S1).

Most variants included in this review (98 out of 121; 81.0%) directly affect essential domains for protein function; these include missense variants directly affecting a specific domain, or variants affecting the whole protein structure (such as frameshift or nonsense) located upstream of reported domains. The specific effect of each mutation on protein function and early fertilization events will be detailed for each factor in the following sections.

Genetic variants in PLCZ1: the sperm-borne oocyte-activating factor

In total, 27 *PLCZ1* exonic variants were identified in 14 studies, including 50 men who experienced FF or low FR after ICSI associated with oocyte activation deficiencies (Table 1). *PLCZ1* is located on chromosome 12, consists of 15 exons, and codes for the sperm-specific enzyme phospholipase C zeta (PLCζ). Since its discovery in 2002 (Saunders *et al.*, 2002), PLCζ has emerged as the main sperm-borne oocyte activation factor (SOAF), a soluble protein conserved through several taxonomic groups that is essential for eliciting the intracellular calcium oscillations observed shortly after the sperm enters the oocyte (Saunders *et al.*, 2002; Coward *et al.*, 2005). In mature sperm, PLCζ localizes in the perinuclear theca, a condensed cytosolic fraction situated between the acrosome and the nuclear envelope, which first contacts the oocyte cytoplasm (Escoffier *et al.*, 2015). Once inside the oocyte, PLCζ functions by hydrolyzing PIP_2 into IP_3 and DAG. Subsequently, PIP_2 is distributed in vesicles throughout the oocyte cytoplasm (Sanders *et al.*, 2018).

The essential role of PLCζ in fertilization has been revealed by numerous findings. Depleting PLCζ from sperm extracts by specific antibodies abolishes the induction of Ca^{2+} oscillations in the oocyte (Saunders *et al.*, 2002). Microinjection of human *PLCZ1* cRNA or recombinant PLCζ protein into human oocytes causes parthenogenetic activation and development to the pseudo-blastocyst stage, inducing Ca^{2+} oscillations that are very similar to those observed after sperm fertilization (Rogers *et al.*, 2004; Yoon *et al.*, 2012). Moreover, recent studies have demonstrated that sperm from *Plcz1* knockout mice did not induce calcium oscillations after ICSI, causing fertilization failure (FF; Hachem *et al.*, 2017; Nozawa *et al.*, 2018).

Approximately a decade ago, the two first *PLCZ1* pathogenic variants associated with FF after ICSI were reported; p.H233L and p.H398P were identified in a non-globozoospermic patient in compound heterozygosity, and were demonstrated to compromise PLCζ function (Heytens *et al.*, 2009; Kashir *et al.*, 2012). Since then, different studies have sequenced *PLCZ1* in men experiencing FF after ICSI (and, in many cases, their close relatives), significantly broadening the list of *PLCZ1* variants with clinical relevance. Of the 27 *PLCZ1* variants compiled in this review, 19 have been reported in the last 3 years (Table 1). While the number of patients carrying *PLCZ1* variants is very low, some small cohort studies may give an idea of the incidence of *PLCZ1* variants among infertile men experiencing FF after ICSI. This incidence is around one-third of the reported cases (33.6%) and seems to be similar across other studies reported in the literature (range 25–35%; Table 2).

Functional and protein structure assays, either experimentally or *in silico*, were performed for 21 out of the 27 (77.8%) *PLCZ1* genetic variants identified so far. Altogether, 18 variants had a demonstrated deleterious effect on PLCζ structure and/or function, one did not cause a negative effect on PLCζ, while two resulted in unclear results (Table 1). In most cases, the frequency of these variants in the general population was below 0.1% (Table S1). However, a higher MAF was reported for some *PLCZ1* variants that were reported to be benign or with unclear effects, such as p.I120M and p.S500L, in accordance with the null or little effect of these genetic variants on fertilization success through ICSI.

As indicated in Table 1 and Figure 2, all *PLCZ1* variants directly affect at least one of the domains required for PLCζ catalytic and regulatory activity, causing a partial or complete failure for the enzyme to generate the calcium oscillations essential for oocyte activation. These domains are tandem EF hands, conferring calcium sensitivity to PLCζ; X and Y core domains, required for

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Table 1. *PLCZ1*, *WBP2NL*, *DNAH17* and *ACTL9* genetic variants detected in non-globozoospermic men presenting fertilization failure or low fertilization rates after ICSI. The table shows the specific exons and protein domains affected, the type of mutation, the dbSNP ID (if any), the gene dosage (Homo: homozygosity, Het: heterozygosity, Het*: compound heterozygosity) and the total number of patients in which the specific variant has been identified. The information about experimental validation for each genetic variant is also indicated, and the colour code provides information on their pathogenicity: no experimental validation reported (grey), reported as benign (green), reported as pathogenic (orange), potentially pathogenic or unclear according to existing data (yellow)

Affected gene	Variant (protein)	Variant (cDNA)	Gene location	Affected protein domain;	Mutation type	dbSNP ID	Dose	Experimental validation	No. patients	Reference/s
PLCZ1	p.D46N	c.136G>A	Exon 4	EF-hand domain	Missense	rs202206940	Het*	No	1	Guggilla *et al.*, 2019
	p.?	c.136−1G>C	Exon 4	EF-hand domain	Splicing	–	Het	No	1	Guggilla *et al.*, 2019
	p.Q94*	c.280C>T	Exon 4	EF-hand domain	Nonsense, stop gain	rs138801851	–	No	1	Ilkan *et al.*, 2014
	p.I120M	c.360C>G	Exon 4	EF-hand domain	Missense	rs79487790	Het	*In silico*, cRNA – Benign	1	Torra-Massana *et al.*, 2019
	p.R141H	c.422G>A	Exon 5	–	Missense	rs202034240	Het	No	1	Guggilla *et al.*, 2019
	p.V189Cfs*12	c.570+1G>T	Exon 5	X catalytic domain	Splicing	–	Het*	*In silico* – Pathogenic	1	Yan *et al.*, 2020
	p.C196*	c.588C>A	Exon 6	X catalytic domain	Nonsense, stop gain	rs535719220	Homo/ Het*	*In silico*, cRNA, WB on recombinant protein –; Pathogenic	6	Dai *et al.*, 2021; Wang *et al.*, 2020; Mu *et al.*, 2020; Yan *et al.*, 2020
	p.R197H	c.590G>A	Exon 6	X catalytic domain	Missense	rs781075636	Homo/ Het*	*In silico*, cRNA, WB on recombinant protein – Pathogenic	3	Ilkan *et al.*, 2014; Torra-Massana *et al.*, 2019; Mu *et al.*, 2020
	p.L224P	c.671T>C	Exon 6	X catalytic domain	Missense	rs144902254	Het	*In silico*, cRNA – Unclear	1	Torra-Massana *et al.*, 2019
	p.H233L	c.698A>T	Exon 6	X catalytic domain	Missense	rs200061726	Het/ Het*	*In silico*, cRNA – Pathogenic	5	Heytens *et al.*, 2009; Kashir *et al.*, 2012; Torra-Massana *et al.*, 2019; Guggilla *et al.*, 2019
	p.L246F	c.736C>T	Exon 7	X catalytic domain	Missense	–	Homo	*In silico* – Potentially pathogenic	1	Dai *et al.*, 2021
	p.L277P	c.830T>C	Exon 7	X catalytic domain	Missense	–	Het*	*In silico*, cRNA – Pathogenic	1	Yan *et al.*, 2020
	p.T303A	c.907A>G	Exon 8	X–Y linker	Missense	–	–	No	1	Ilkan *et al.*, 2014
	p.K322*	c.964A>T	Exon 9	X–Y linker	Nonsense, stop gain	–	Het/ Het*	*In silico*, cRNA – Pathogenic	2	Amdani *et al.*, 2016; Guggilla *et al.*, 2019
	p.V326Kfs*25	c.972_973delAG	Exon 9	X–Y linker	Frameshift deletion	rs777169092	Het/ Het*	*In silico*, cRNA, WB on recombinant protein – Pathogenic	2	Torra-Massana *et al.*, 2019; Mu *et al.*, 2020
	p.S350P	c.1048 T>C	Exon 10	Y catalytic domain	Missense	–	Homo	*In silico* – Potentially pathogenic	1	Dai *et al.*, 2021

(*Continued*)

Table 1. (*Continued*)

Affected gene	Variant (protein)	Variant (cDNA)	Gene location	Affected protein domain;	Mutation type	dbSNP ID	Dose	Experimental validation	No. patients	Reference/s
	p.N377del	c.1129_1131delAAT	Exon 10	Y catalytic domain	In-frame deletion	rs758499880	Het*	*In silico*, cRNA – Pathogenic	1	Yan *et al.*, 2020
	p.A384V	c.1151C>T	Exon 10	Y catalytic domain	Missense	rs1334320857	Homo	*In silico*, cRNA – Pathogenic	1	Yan *et al.*, 2020
	p.H398P	c.1193A>C	Exon 11	Y catalytic domain	Missense	rs1359777791	Het*	In silico, cRNA – Pathogenic	1	Heytens *et al.*, 2009; Kashir *et al.*, 2012
	p.R412Efs*15	c.1234delA	Exon 11	Y catalytic domain	Frameshift deletion	rs1165227004	Het*	*In silico*, cRNA, WB on recombinant protein – Pathogenic	1	Mu *et al.*, 2020
	p.P420L	c.1259C>T	Exon 11	Y catalytic domain	Missense	–	Het*	*In silico*, cRNA, WB on recombinant protein, analysis of PLC catalytic activity *in vitro* – Pathogenic	2	Mu *et al.*, 2020; Yuan *et al.*, 2020 (b)
	p.L440R	c.1319T>G	Exon 12	Y catalytic domain	Missense	–	–	No	1	Ilkan *et al.*, 2014
	p.K448N	c.1344A>T	Exon 12	Y catalytic domain	Missense	–	Het*	*In silico*, cRNA – Pathogenic	1	Yan *et al.*, 2020
	p.I489F	c.1465A>T	Exon 13	C2 domain	Missense	rs757326350	Homo	*In silico*, cRNA – Pathogenic	2	Escoffier *et al.*, 2016
	p.S500L	c.1499C>T	Exon 13	C2 domain	Missense	rs10505830	Homo/ Het/ Het*	*In silico*, cRNA – Unclear	18	Yoon *et al.*, 2008; Ilkan *et al.*, 2014; Torra-Massana *et al.*, 2019; Guggilla *et al.*, 2019
	p.R553P	c.1658G>C	Exon 14	C2 domain	Missense	–	Homo	*In silico*, cRNA – Pathogenic	1	Yuan *et al.*, 2020 (a)
	p.M578T	c.1733T>C	Exon 14	C2 domain	Missense	rs758723831	Het*	*In silico*, cRNA, analysis of PLC catalytic activity *in vitro* – Pathogenic	2	Yan *et al.*, 2020; Yuan *et al.*, 2020 (b)
WBP2NL	p.Q5E	c.13C>G	Exon 1	–	Missense	rs17002790	Homo	No	1	Freour *et al.*, 2018
	p.D121G	c.362A>G	Exon 4	–	Missense	rs133335	Homo/ Het	No (Benign)	4*	Freour *et al.*, 2018
	p.C170F	c.409G>T	Exon 5	–	Missense	rs17002802	Homo	No	1	Freour *et al.*, 2018
	p.Q285H	c.855G>C	Exon 6	–	Missense	rs2301521	Homo	No (Benign)	2*	Freour *et al.*, 2018
DNAH17	p.R350*	c.1048C>T	Exon 7	STEM	Nonsense, stop gain	rs371400048	Het*	No	1	Jia *et al.*, 2021
	p.M1130I	c.3390G>A	Exon 22	STEM	Missense	rs189737946	Het*	No	1	Jia *et al.*, 2021
ACTL9	p.S345L	c.1034C>T	Exon 1	–	Missense	rs1478948010	Homo	*In silico*, analysis of sperm ultrastructure by TEM, ACTL9 and PLCZ1 immunostaining, analysis of Ca^{2+} oscillations in the oocyte, analysis of *Actl9*-mutated male mice – Pathogenic	1	Dai *et al.*, 2021
	p.V380L	c.1138G>T	Exon 1	–	Missense	rs532021673	Homo	*In silico*, analysis of sperm ultrastructure by TEM, ACTL9 and PLCZ1 immunostaining, analysis of Ca^{2+} oscillations in the oocyte, analysis of *Actl9*-mutated male mice – Pathogenic	1	Dai *et al.*, 2021

Table 1. (*Continued*)

	p.Y403*	c.1209C>G	Exon 1	–	Nonsense, stop gain	rs34687433	Homo	*In silico*, analysis of sperm ultrastructure by TEM, ACTL9 and PLCZ1 immunostaining, analysis of Ca^{2+} oscillations in the oocyte, analysis of *Actl9*-mutated male mice – Pathogenic	1	Dai *et al.*, 2021
ACTL7A	p.R155*	c.463C>T	Exon 1	–	Nonsense, stop gain	rs755704105	Het*	*In silico*, analysis of sperm ultrastructure by TEM, PLCZ1 immunostaining – Potentially pathogenic	1	Wang *et al.*, 2021
	p.G362R	c.1084G>A	Exon 1	–	Missense	rs779515458	Het*	*In silico*, analysis of sperm ultrastructure by TEM, PLCZ1 immunostaining – Potentially pathogenic	1	Wang *et al.*, 2021

enzymatic catalytic activity; the C2 domain, essential for PLCζ to bind to two phosphoinositides (PI(3)P and PI(5)P) present in liposomes, therefore facilitating substrate – $PI(4,5)P_2$ – accessibility; and the XY-linker region, involved in both enzymatic and regulatory functions (Saunders *et al.*, 2002; Kouchi *et al.*, 2005; Nomikos *et al.*, 2011, 2017).

Most *PLCZ1* variants affect the catalytic domains X and Y (15; 55.6%). As previously demonstrated, missense variants affecting the C2 domain (such as the p.I489F) can affect PLCζ ability to bind to liposomes containing its substrate (Nomikos *et al.*, 2017) and, perhaps due to the regulatory nature of this domain, the *in vitro* effect of these variants can be compensated by increasing the amount of mutated PLCζ. For instance, for p.I489F and p.R553P variants, microinjection of physiological levels of mutated cRNA failed to trigger calcium oscillations in mice oocytes, but a two-fold increase in the same cRNA led to successful oocyte activation (Nomikos *et al.*, 2017; Yuan *et al.*, 2020). In contrast, mutations such as p.V326Kfs*25 and p.R412Efs*15 resulted in truncated and completely non-functional forms of PLCζ that lacked the C2 domain and part of the catalytic domain (Torra-Massana *et al.*, 2019; Mu *et al.*, 2020). Regarding the EF hands domains, no variants identified in this region have been proven as pathogenic by *in vitro* experimental procedures, and one of them (p.I120M) was characterized as benign; while this region helps PLCζ gain its enzymatic activity by binding calcium, its role is not critical in fertilization through ICSI.

An altered enzymatic function is not the only alteration caused by *PLCZ1*, as this problem is usually accompanied by abnormal levels and subcellular localization of PLCζ protein (Escoffier *et al.*, 2016; Wang *et al.*, 2020; Yan *et al.*, 2020), often confirmed in somatic cells transfected with mutant *PLCZ1* (Mu *et al.*, 2020). Nevertheless, some patients carrying *PLCZ1* variants with apparently normal levels and subcellular localization of PLCζ are reported to experience FF even in ICSI cycles using fertility-proven oocytes from donors (Torra-Massana *et al.*, 2019). It is tempting to speculate that the effect of *PLCZ1* variants on protein expression and subcellular localization could be specific for each variant depending on additional unknown factors. The differences observed between studies could be attributed to the large variability in levels and localization of PLCζ among patients, even in normozoospermic men with good FR (Ferrer-Vaquer *et al.*, 2016).

Additional genetic variants associated with male-related fertilization failure

PLCZ1 is not the only gene that has been associated with FF; for example, this is also the case for *WBP2NL*. *WBP2NL* gene is located on chromosome 22 in humans, consists of six exons and codes for the post-acrosomal WW domain-binding protein (PAWP), a sperm-specific protein expressed in elongated spermatids and located in the post-acrosomal sheath in the mature sperm (Wu *et al.*, 2007). PAWP was reported to promote meiotic resumption and pronuclear formation when injected in porcine, bovine, macaque, and *Xenopus* oocytes (Wu *et al.*, 2007). PAWP contains PPXY motifs that would interact with the WW domain present in PLCϒ, in turn hydrolyzing PIP_2 and triggering oocyte activation following a non-canonical pathway (Aarabi *et al.*, 2014). However, the PAWP role in human oocyte activation is not clear, as PLCϒ is not required to generate calcium oscillations in the human oocyte (Kline *et al.*, 1999). While some reports propose that PAWP (either cRNA or recombinant protein) can trigger calcium oscillations and oocyte activation (Aarabi *et al.*, 2014), other

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Table 2. List of studies screening for *PLCZ1* or *WEE2* genetic variants in multiple male or female patients (≥3 unrelated individuals) who experienced fertilization failure or low fertilization rates after ICSI. The population of patients as defined in each study is indicated. The frequency of patients carrying at least one variant in *PLCZ1* or *WEE2* among all FF patients is indicated for each study and overall

PLCZ1				
Reference	Population of patients	Total patients screened	Patients with *PLCZ1* variant	%
Yoon *et al.*, 2008	Fertilization failure or low fertilization rate (<25%) after ICSI	3	1	33
Heytens *et al.*, 2009	Fertilization failure after ICSI due to sperm defects verified by MOAT	4	1	25
Torra-Massana *et al.*, 2019	Fertilization failure or low fertilization rate (≤25%) after ICSI	37	13	35.1
Guggilla *et al.*, 2019	Fertilization failure or low fertilization rate after ICSI (<33%)	35	12	34.3
Dai *et al.*, 2021	Fertilization failure or low fertilization rate after ICSI (<20%)	10	3	30
Wang *et al.*, 2020	Fertilization failure after ICSI	4	1	25
Yan *et al.*, 2020	Fertilization failure or low fertilization rate (<25%) after ICSI	14	5	35.7
	TOTAL:	107	36	33.6
WEE2				
Reference	Population of patients	Total patients screened	Patients with *WEE2* variant	%
Sang *et al.*, 2018; Zhang *et al.*, 2019	Total fertilization failure after ICSI	25	10	40
Dai *et al.*, 2019	Fertilization failure or low fertilization rate after ICSI (<20%)	24	5	20.8
Zhao *et al.*, 2019	Fertilization failure after ICSI	90	5	5.6
Zhou *et al.*, 2019	Fertilization failure after ICSI	17	1	5.9
	TOTAL:	156	21	13.5

authors failed to confirm these findings when repeating the same experiment in mouse oocytes (Nomikos *et al.*, 2014). Additional data reported normal fertility in *Wbp2nl*$^{-/-}$ mice (Satouh *et al.*, 2015).

Nonetheless, some attempts have been made to find the genetic alterations in *WBP2NL* associated with FF after ICSI. Two studies did not find *WBP2NL* variants in patients with FF after ICSI (Escoffier *et al.*, 2016; Wang *et al.*, 2020). Freour and colleagues found four different missense variants in *WBP2NL* in six patients with FF after ICSI (Freour *et al.*, 2018). Two of these variants (p.D121G and p.Q285H) were not likely to affect sperm fertilization ability, as they were also found in men with good FR, while two variants (p.Q5E and p.C170F) were identified in homozygosity exclusively in FF patients (Table 1). As no functional tests were performed, the effect of these variants on sperm fertilization ability remains uncertain. The reported MAF for all four *WBP2NL* gene variants indicates that its frequency is very high in the general population, reinforcing the idea that these common genetic variants do not affect the sperm's ability to trigger oocyte activation (Table S1).

PAWP protein contains a C-terminal region rich in proline, with a PPXY consensus sequence that can interact with WW group I domain-containing proteins, as well as a repeated motif (YGXPPXG), and a GRAM domain in the N-terminus (Wu *et al.*, 2007). However, none of the two variants detected exclusively in FF patients (p.Q5E and p.C170F) affects these domains directly (Figure 2). So far, contrary to what happens with PLCζ, the role of PAWP in human fertilization has not been demonstrated and should not be considered a diagnostic target; and the same seems to be true for WBP2 (the ortholog of *WBP2NL*) (Hamilton *et al.*, 2018).

Recently, three additional genes have broadened the spectrum of factors associated with male-related FF after ICSI, historically limited to *PLCZ1*. The first one is *ACTL9*, which encodes actin-like protein 9. Dai and colleagues reported three infertile patients carrying *ACTL9* pathogenic variants in homozygosity, all of them experiencing total FF due to OAF after ICSI (Dai *et al.*, 2021; Table 1). These variants led to ultrastructural defects in the sperm as analyzed by TEM, in which the acrosome is detached from the nuclear envelope causing perinuclear theca abnormalities. Apparently, mutant sperm lose the interaction between ACTL9 and ACTL7A (its paralog, another actin-like protein required to maintain the ultrastructure of the acrosome), which led to lower levels and altered localization of PLCζ within the sperm head, therefore explaining the inability to trigger calcium oscillations (Dai *et al.*, 2021). For this reason, *ACTL7A* could be considered as an additional male-related FF target and, indeed, two variants in compound heterozygosity were recently identified in this gene in a patient with FF after ICSI (Wang *et al.*, 2021). Interestingly, *ACTL7A* genetic variants can also cause acrosomal defects and altered PLCζ localization.

Finally, two genetic variants affecting the *DNAH17* gene were identified in a compound heterozygous infertile man who experienced FF after ICSI and ICSI-AOA (Jia *et al.*, 2021). *DNAH17* encodes dynein axonemal heavy chain 17, a protein specifically located in the sperm flagellum. Inner and outer dynein arm proteins are essential for sperm motility as they are structural components of the axoneme and allow beating of the sperm flagellum. Both variants were reported by Jia and colleagues, the first case report associating this kind of variant with FF, affecting the STEM domain (Table 1 and Figure 2). At least for the p.R350* variant, a truncated non-functional form of the protein is predicted; however, no functional tests were performed and further research is needed to characterize the effect of these variants on protein function and the FF phenotype.

Previous reports have associated the presence of *DNAH1*, *DNAH2*, *DNAH7*, *DNAH8* and *DNAH10* mutations (other dynein arm proteins) with asthenoteratozoospermia and multiple morphological abnormalities of the flagella (MMAF), but the ICSI outcome was successful (Wambergue *et al.*, 2016; Liu *et al.*, 2020; Gao *et al.*, 2021; Tu *et al.*, 2021; Wei *et al.*, 2021). Regarding *DNAH17*, several genetic variants in these genes have been previously associated with poor ICSI outcomes (failure to achieve pregnancy;

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

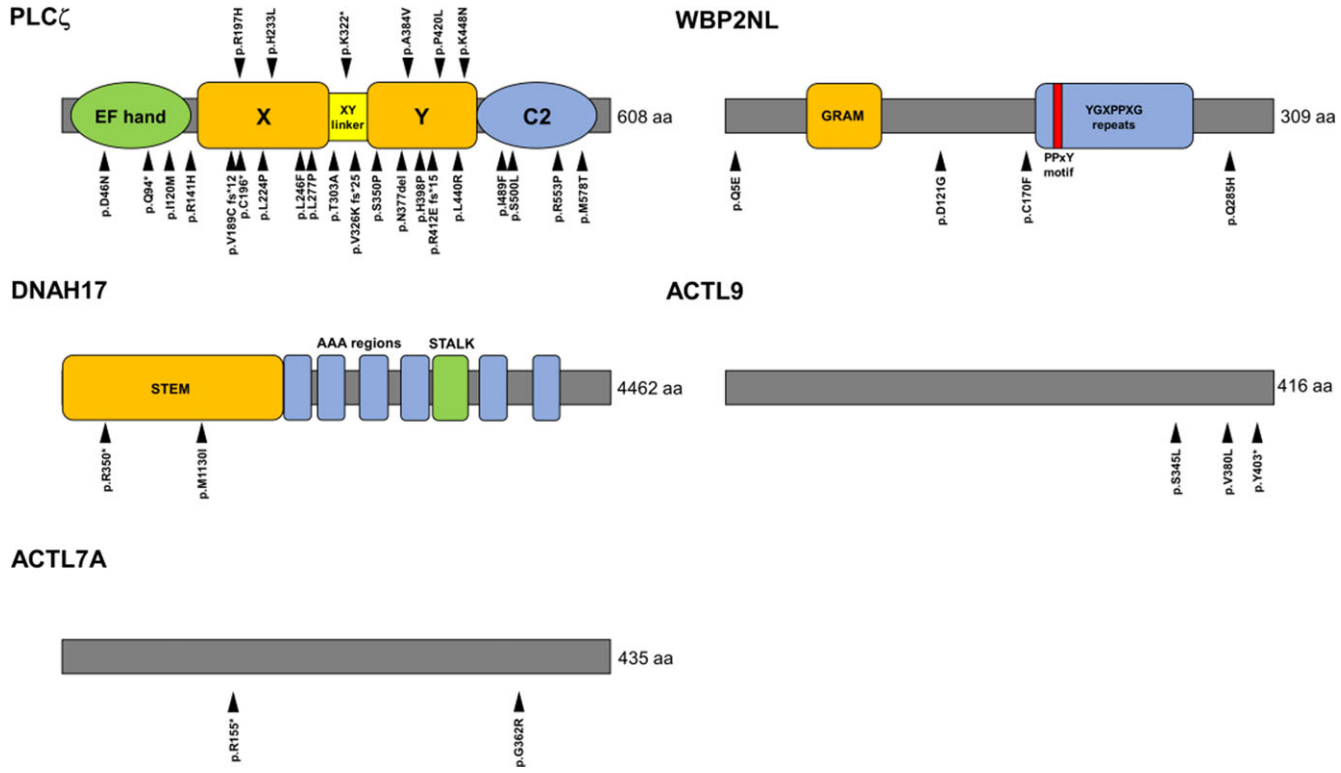

Figure 2. Location of genetic variants associated with male-related fertilization failure within the protein structure. The main domains and the total number of amino acids are indicated for each protein.

Whitfield *et al.*, 2019). Recently, a *DNAH17* variant in homozygosity was reported in a patient with MMAF, but fertilization after ICSI was successful (Zheng *et al.*, 2021a). These observations suggest that, despite different *DNAH17* variants being associated with asthenozoospermia, the sperm fertilization ability by ICSI and its downstream outcomes are variable. In addition, while not substantially affecting the early fertilization events, other genes associated with MMAF such as *FSIP2* or *CFAP65* may compromise embryo development and ICSI outcomes (Wang *et al.*, 2019; Liu *et al.*, 2021).

WEE2: the main factor explaining female-related OAF

WEE2 encodes an oocyte activation factor in which several variants have been identified; specifically, 26 variants carried by 24 infertile women presenting FF or low FR after ICSI due to oocyte activation deficiencies (Table 3). *WEE2* is located on chromosome 7, consists of 12 exons and codes for Wee1-like protein kinase 2 (WEE2, also called Wee1B). WEE2 is a cytoplasmic kinase responsible for maintaining meiotic arrest at the GV stage by inactivating MPF and it is also essential for MII exit (Oh *et al.*, 2010, 2011). Phosphorylation and activation of WEE2 (by CAMKII) and WEE2-mediated phosphorylation of Cdc2 are essential for oocyte activation and subsequent formation of pronuclei, respectively (Oh *et al.*, 2011). For this reason, WEE2 deleterious variants cause the failure of pronuclei formation and FF after ICSI, a phenotype rescued by injection of wild-type *WEE2* cRNA (Sang *et al.*, 2018). By either *in silico* or experimental protein structure or function assays, 25 out of 26 *WEE2* variants identified so far (96.2%) in infertile women are considered pathogenic or potentially pathogenic (Table 3). The mean proportion of infertile women experiencing FF due to OAF carrying *WEE2* variants may be ~13.5% (Table 2), but this proportion may not reflect the real incidence of these variants, as it is calculated from a few case reports.

The severity of *WEE2* pathogenic variants is diverse; while frameshift variants seem to produce a dramatic effect on oocyte activation ability, resulting in total FF after ICSI, in other cases residual fertilization can occur (Zhang *et al.*, 2019). Similarly to the outcome for PLCζ, some reports have associated the presence of variants with lower expression levels of the protein and alterations in its subcellular localization within the oocyte or in transfected cells, something that can explain the FF phenotype (Sang *et al.*, 2018; Zhou *et al.*, 2019).

The primary effect of *WEE2* exonic variants is a dysfunction of kinase activity. WEE2 protein contains a Pkinase domain essential for this activity, a domain directly affected by 15 out of 26 (57.7%) of *WEE2* variants compiled in the present review (Figure 3). Some variants (such as p.R207C) are predicted to destroy the hydrogen bonds between residues crucial for WEE2 function (Yang *et al.*, 2019). Concomitantly, variants such as p.E75Vfs*6, p.D234H and p.H337Yfs*24 compromise WEE2-dependent phosphorylation of Cdc2, preventing meiotic resumption and causing FF (Sang *et al.*, 2018).

TUBB8: a factor not only associated with oocyte maturation defects

One of the genes with the most genetic variants explaining female-related zygotic problems after ICSI is *TUBB8*. In total, 27 *TUBB8* variants present in 33 infertile women were identified in six studies (Table 4). Historically, the most frequent phenotype associated with *TUBB8* sequence variants has been oocyte maturation arrest (either at the GV or MI stage) leading to the absence of MII oocytes (Feng *et al.*, 2016; Yuan *et al.*, 2018). However, as shown in Table 4,

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Table 3. *WEE2* genetic variants detected in women presenting fertilization failure or low fertilization rates after ICSI. The table indicates the specific exons and protein domains affected, the type of variant, the dbSNP nomenclature (if any), the gene dosage (Homo: homozygosity, Het: heterozygosity, Het*: compound heterozygosity) and the total number of patients in which the specific variant has been identified. The information about experimental validation for each genetic variant is also indicated, and the colour code provides information of their pathogenicity: no experimental validation reported (grey), reported as benign (green), reported as pathogenic (orange), potentially pathogenic or unclear according to existing data (yellow).

Variant (protein)	Variant (cDNA)	Gene location	Affected protein domain	Mutation type	dbSNP ID	Dose	Experimental validation	Nº patients	Reference/s
p.0?	c.1A>G	Exon 1	–	Frameshift/ Nonsense	–	Het*	*In silico* – Deleterious	1	Zhao *et al.*, 2019
p.E75Vfs*6	c.224_227delAAAG	Exon 1	–	Frameshift deletion	rs768323979	Homo/ Het*	*In silico*, subcellular localization in transfected cells, localization of mutant WEE2 in mouse GV oocytes, WB on transfected cells – Deleterious	3	Sang *et al.*, 2018; Zhang *et al.*, 2019
p.P98Pfs*2	c.293_294insCTGAGACACCAGCCCAACC	Exon 1	–	Frameshift insertion	–	Homo	*In silico* – Deleterious	1	Zhao *et al.*, 2019
p.K114Nfs*20	c.341_342delAA	Exon 1	–	Frameshift deletion	–	Het*	*In silico* – Deleterious	1	Zhao *et al.*, 2019
p.K195N	c.585G>C	Exon 3	–	Missense	rs1448207084	Homo	*In silico* – Potentially deleterious	1	Dai *et al.*, 2019
p.R200*	c.598C>T	Exon 4	–	Nonsense, stop gain	rs754062320	Het*	Subcellular localization in transfected cells, WB on transfected cells – Deleterious	2	Zhang *et al.*, 2019; Zhou *et al.*, 2019
p.R207C	c.619C>T	Exon 4	–	Missense	rs200326581	Homo	*In silico* – Potentially deleterious	1	Yang *et al.*, 2019
p.E209*	c.625G>T	Exon 4	–	Nonsense, stop gain	–	Het*	*In silico* – Potentially deleterious	1	Wang *et al.*, 2021
p.D234H	c.700G>C	Exon 4	Pkinase	Missense	rs1554415096	Homo	*In silico*, subcellular localization in transfected cells, localization of mutant WEE2 in mouse GV oocytes, WB on transfected cells – Deleterious	1	Sang *et al.*, 2018
p.R242P	c.725G>C	Exon 4	Pkinase	Missense	–	Het*	*In silico* – Potentially deleterious	1	Zhang *et al.*, 2019
p.Q288H	c.864G>C	Exon 5	Pkinase	Missense	rs973812791	Het*	*In silico* – Potentially deleterious	1	Zhao *et al.*, 2019
p.L316F	c.946C>T	Exon 6	Pkinase	Missense	rs371571990	Het*	*In silico* – Potentially deleterious	1	Tian *et al.*, 2020
p.H331N	c.991C>A	Exon 6	Pkinase	Missense	–	Het*	*In silico* – Potentially deleterious	1	Zhao *et al.*, 2019
p.S333P	c.997T>C	Exon 6	Pkinase	Missense	–	Het*	*In silico* – Potentially deleterious	1	Zhang *et al.*, 2019
p.H337Yfs*24	c.1007_1008dupTA	Exon 6	Pkinase	Frameshift insertion	rs1370452353	Homo/ Het*	Oocyte WEE2 immunofluorescence, subcellular localization in transfected cells, localization of mutant WEE2 in mouse GV oocytes, WB on transfected cells – Deleterious	5	Sang *et al.*, 2018; Zhang *et al.*, 2019; Dai *et al.*, 2019
p.G379Efs*6	c.1136−2A>G	Exon 6	Pkinase	Splicing	–	Het*	Oocyte *WEE2* RNA reverse transcription + sequencing – Deleterious	1	Dai *et al.*, 2019
p.G395E	c.1184G>A	Exon 8	Pkinase	Missense	–	Homo	*In silico* – Potentially deleterious	1	Zhang *et al.*, 2019
p.D408Vfs*1	c.1221G>A	Exon 8	Pkinase	Splicing	–	Het*	cDNA sequencing – Deleterious	1	Zhang *et al.*, 2019

Table 3. (*Continued*)

p.R410W	c.1228C>T	Exon 9	Pkinase	Missense	rs905047580	Homo	*In silico* – Potentially deleterious	2	Zhang *et al.*, 2019; Dai *et al.*, 2019
p.G421R	c.1261G>A	Exon 9	Pkinase	Missense	–	Het*	*In silico* – Potentially deleterious	1	Zhao *et al.*, 2019
p.G429del	c.1286_1288delGAG	Exon 9	Pkinase	In-frame deletion	–	Het*	*In silico* – Potentially deleterious	1	Zhang *et al.*, 2019
p.T435Mfs*31	c.1304_1307delCCAA	Exon 9	Pkinase	Frameshift deletion	–	Het*	*In silico* – Deleterious	1	Zhao *et al.*, 2019
p.W440S	c.1319G>C		Pkinase	Missense	–	Het*	Subcellular localization in transfected cells, WB on recombinant protein – Deleterious	1	Zhou *et al.*, 2019
p.T493Nfs*39	c.1477_1478insA	Exon 10	–	Frameshift insertion	rs1554416415	Homo	Subcellular localization in transfected cells, localization of mutant WEE2 in mouse GV oocytes, WB on transfected cells – Deleterious	1	Sang *et al.*, 2018
p.?	c.1535+3A>G	Exon 10	–	Splicing	–	Het*	*In silico* – Potentially deleterious	1	Tian *et al.*, 2020
p.Y526D	c.1576T>G	Exon 11	–	Missense	rs35683659	Homo	*In silico* – Potentially benign	1	Zhao *et al.*, 2019

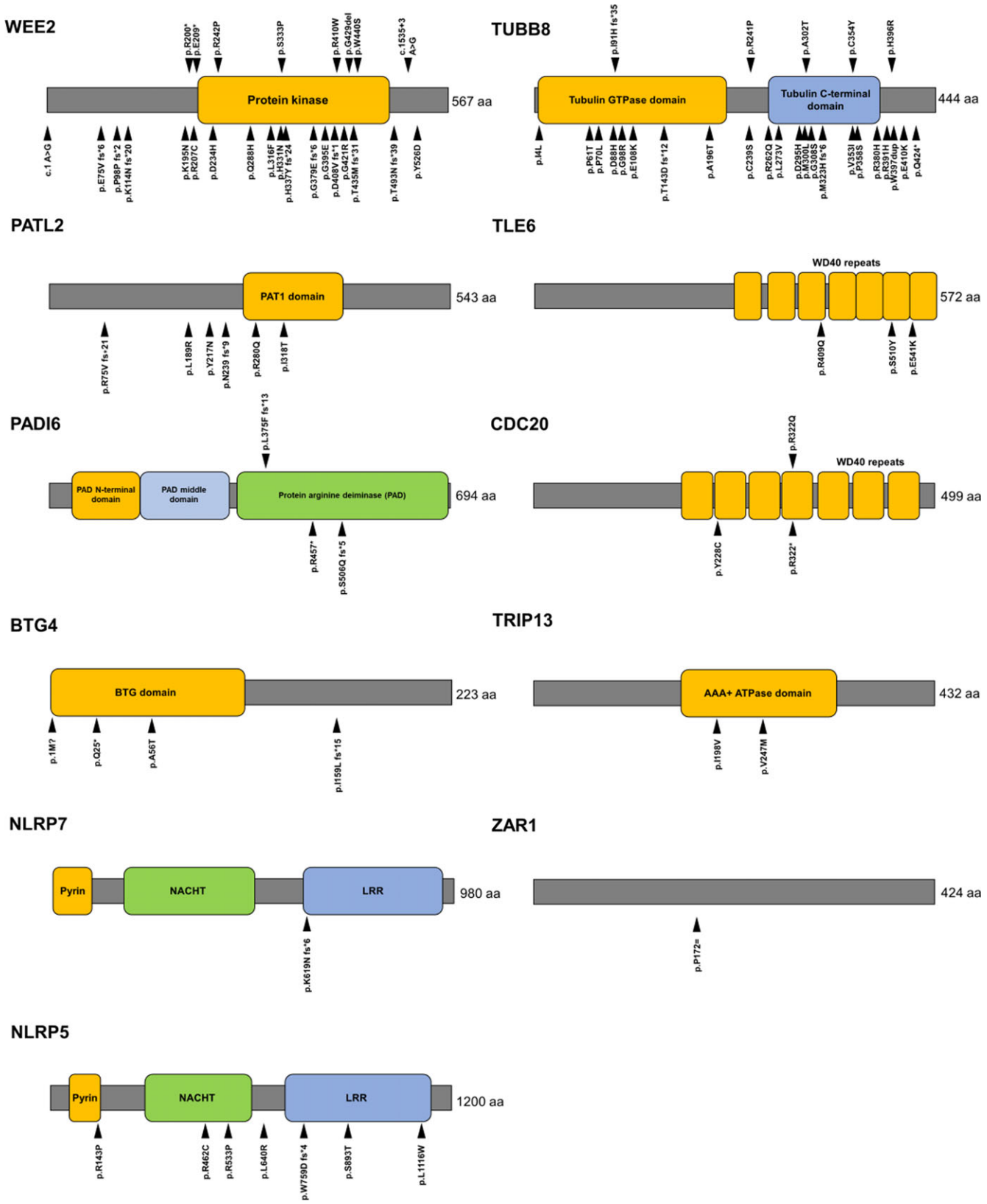

Figure 3. Location of genetic variants associated with female-related fertilization failure within the protein structure. The main domains and the total number of amino acids are indicated for each protein.

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Table 4. *TUBB8* genetic variants detected in women presenting fertilization failure, low fertilization rates abnormal fertilization and/or cleavage failure after ICSI. The table indicates the specific exons and protein domains affected, the type of variant, the dbSNP nomenclature (if any), the gene dosage (Homo: homozygosity, Het: heterozygosity, Het*: compound heterozygosity) and the total number of patients in which the specific variant has been identified. The information about experimental validation for each genetic variant is also indicated, and the colour code provides information of their pathogenicity: no experimental validation reported (grey), reported as benign (green), reported as pathogenic (orange), potentially pathogenic or unclear according to existing data (yellow). *Variants also observed in other patients exhibiting total oocyte maturation arrest or embryo developmental arrest

Variant (protein)	Variant (cDNA)	Gene location	Mutation type	Affected protein domain	dbSNP ID	Dose	Experimental validation	Nº patients	Phenotype/s	Reference/s
p.I4L	c.10A>C	Exon 1	Missense	GTPase	rs199817418	Het	*In silico* – Potentially benign	1	Oocyte maturation arrest, FF, zygotic arrest	Chen *et al.*, 2017a; Chen *et al.*, 2019*
p.P61T	c.181C>A	Exon 3	Missense	GTPase	–	Het	*In silico* – Potentially pathogenic	1	FF, cleavage failure	Chen *et al.*, 2019
p.P70L	c.209C>T	Exon 3	Missense	GTPase	rs782247878	Homo	*In silico* – Potentially pathogenic	1	FF	Chen *et al.*, 2017a
p.D88H	c.262G>C	Exon 3	Missense	GTPase	–	Het	*In silico* – Potentially pathogenic	1	FF, abnormal fertilization	Zheng *et al.*, 2021a
p.I91Hfs*35	c.269dupT	Exon 3	Frameshift insertion	GTPase	–	Het*	*In silico* – Potentially pathogenic	1	FF	Zheng *et al.*, 2021a
p.G98R	c.292G>A	Exon 4	Missense	GTPase	rs1339474843	Het	*In silico* – Potentially pathogenic	1	FF	Zheng *et al.*, 2021a
p.E108K	c.322G>A	Exon 4	Missense	GTPase	rs541770879	Homo/ Het*	*In silico* – Potentially pathogenic	4	FF, zygotic arrest	Yuan *et al.*, 2018; Chen *et al.*, 2019; Zheng *et al.*, 2021a
p.T143Dfs*12	c.426dupG	Exon 4	Frameshift insertion	GTPase	rs782683305	Het*	*In silico* – Potentially pathogenic	2	FF, zygotic arrest	Chen *et al.*, 2019; Zheng *et al.*, 2021a
p.A196T	c.586G>A	Exon 4	Missense	GTPase	rs1554738381	Het	*In silico* – Unclear	1	FF, abnormal fertilization	Zheng *et al.*, 2021a
p.C239S	c.716G>C	Exon 4	Missense	–	–	Het	*In silico* – Potentially pathogenic	1	FF	Zhao *et al.*, 2021
p.R241P	c.722G>C	Exon 4	Missense	–	rs782631950	Het*	*In silico* – Potentially pathogenic	1	Abnormal fertilization	Zheng *et al.*, 2021a
p.R262Q	c.785G>A	Exon 4	Missense	C-terminal	rs869025610	Het	*In silico* – Potentially pathogenic	1	Zygotic arrest, FF	Zheng *et al.*, 2021a
p.L273V	c.817C>G	Exon 4	Missense	C-terminal	–	Het	*In silico*, expression of mutant protein in HeLa cells – Potentially pathogenic	1	FF	Liu *et al.*, 2021
p.D295H	c.883G>C	Exon 4	Missense	C-terminal	–	Het	*In silico* – Potentially pathogenic	1	FF	Chen *et al.*, 2019
p.M300L	c.898A>T	Exon 4	Missense	C-terminal	–	Het	*In silico* – Potentially pathogenic	2	FF	Zheng *et al.*, 2021a
p.A302T	c.904G>A	Exon 4	Missense	C-terminal	–	Het	*In silico* – Potentially pathogenic	1	Abnormal fertilization	Zheng *et al.*, 2021a
p.G308S	c.922G>A	Exon 4	Missense	C-terminal	rs782575307	Homo	*In silico* – Potentially pathogenic	2	FF, zygotic arrest	Zheng *et al.*, 2021a
p.M323Hfs*6	c.966dupC	Exon 4	Frameshift insertion	C-terminal	–	Het*	*In silico* – Potentially pathogenic	1	Zygotic arrest	Zheng *et al.*, 2021a
p.V353I	c.1057G>A	Exon 4	Missense	C-terminal	–	Het	*In silico* – Potentially benign	3	FF, zygotic arrest	Chen *et al.*, 2019; Zheng *et al.*, 2021a

(*Continued*)

Table 4. (*Continued*)

Variant (protein)	Variant (cDNA)	Gene location	Mutation type	Affected protein domain	dbSNP ID	Dose	Experimental validation	Nº patients	Phenotype/s	Reference/s
p.C354Y	c.1061G>A	Exon 4	Missense	C-terminal	–	Het	*In silico* – Potentially pathogenic	1	Oocyte maturation arrest, FF	Chen *et al.*, 2019
p.P358S	c.1072C>T	Exon 4	Missense	C-terminal	–	Het	*In silico* – Potentially pathogenic	1	Zygotic arrest	Zheng *et al.*, 2021a
p.R380H	c.1139G>A	Exon 4	Missense	C-terminal	rs1554738022	Het	*In silico* – Potentially pathogenic	1	Zygotic arrest	Zhao *et al.*, 2021*
p.R391H	c.1172G>A	Exon 4	Missense	–	–	Het	*In silico* – Potentially pathogenic	3	Oocyte maturation arrest, FF, zygotic arrest	Zhao *et al.*, 2021; Zheng *et al.*, 2021a *
p.H396R	c.1187A>G	Exon 4	Missense	–	–	Het	*In silico* – Potentially pathogenic	1	Zygotic arrest	Zhao *et al.*, 2021
p.W397dup	c.1190_1192dup	Exon 4	In-frame insertion	–	–	Het*	No	1	Abnormal fertilization	Zheng *et al.*, 2021a
p.E410K	c.1228G>A	Exon 4	Missense	–	–	Het	*In silico* – Unclear	1	Oocyte maturation arrest, FF	Chen *et al.*, 2019
p.Q424*	c.1270C>T	Exon 4	Nonsense, stop gain	–	rs1356357815	Homo	No	1	FF	Chen *et al.*, 2019

genetic alterations in *TUBB8* display a wide range of phenotypes including FF, low FR, abnormal fertilization and also zygotic arrest (PN are formed but the first mitotic division does not occur). Different studies have estimated that ~30–40% of infertile women experiencing oocyte maturation arrest may present genetic variants in the *TUBB8* gene (Feng *et al.*, 2016; Chen *et al.*, 2017a; Chen *et al.*, 2019; Yang *et al.*, 2021). Variants associated with this phenotype or embryo developmental arrest at the cellular stage were not included in this review.

Microtubules, part of the cell cytoskeleton, are polymers formed by α- and β-tubulin heterodimers. These filaments constitute the meiotic spindle and are essential for oocyte maturation, but they are also involved in early fertilization events such as the extrusion of the second polar body and the first mitotic division. *TUBB8* codes for β-tubulin isotype 8 (one of the nine β-tubulin isotypes), the main constituent of the oocyte (from the GV to MII stages) and embryo spindles. Therefore, genetic alterations of *TUBB8* may result in irregular arrangements of the spindles and compromise the meiotic divisions in the oocyte or the first mitotic division in the zygote (Chen *et al.*, 2019). Indeed, 21 out of 27 variants compiled (77.8%) are considered pathogenic or potentially pathogenic (Table 4).

TUBB8 variants are associated with a wide spectrum of phenotypes, but the molecular alterations behind them are similar. *TUBB8* variants can affect the folding and stability of β-tubulin, and reduce or prevent the interaction between α- and β-tubulin, causing problems in α/β heterodimer assembly and microtubule dynamics (Feng *et al.*, 2016). These problems result in the disrupted organization of cytoplasmic microtubules and spindles with aberrant morphology, altogether compromising oocyte maturation and early mitotic divisions (Feng *et al.*, 2016). As shown in Figure 3, most *TUBB8* variants directly affect the GTPase domain and the C-terminal domain, potentially affecting protein function.

It is unclear which molecular mechanisms explain why some *TUBB8* alterations are exclusively associated with total oocyte maturation arrest or embryo arrest at the cleavage stage (more than 50, data not shown), while others cause FF or zygotic arrest, and for some variants (such as p.E108K, p.T143Dfs*12, p.D295H, as some examples) the vast majority of retrieved oocytes were morphologically MII (Yuan *et al.*, 2018; Chen *et al.*, 2019). The phenotypic variability observed for the same TUBB8 variant could be explained by variable penetrance (Castel *et al.*, 2018).

Additional genetic variants associated with female-related FF and zygotic arrest

In addition to *WEE2* or *TUBB8*, other genetic variants have been identified in infertile women in nine more genes affecting early fertilization events, all of them reported in Table 5. For example, two recent studies have identified mutations affecting *CDC20* in two infertile women in homozygosity or compound heterozygosity, both presenting FF or low FR after ICSI (Xu *et al.*, 2021; Zhao *et al.*, 2021). Apart from these women, described in Table 5, *CDC20* variants have been identified in patients experiencing complete oocyte maturation arrest at the MI stage or embryonic arrest (Huang *et al.*, 2021; Zhao *et al.*, 2021). CDC20 is a cell division cycle protein that acts as an activator of the anaphase-promoting complex/cyclosome (APC/C), enabling the degradation of cyclin B1 and promoting the start of anaphase (Hwang *et al.*, 1998); based on that, one would expect an incomplete occurrence of meiosis in cases in which CDC20 is non-functional within the oocyte: oocyte maturation arrest or failure to resume meiosis after sperm entry, as

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Table 5. Genetic variants affecting different genes identified in patients with fertilization failure or low fertilization rates after ICSI, abnormal fertilization, oocyte degeneration or zygotic arrest at the pronuclear stage. The affected gene, specific protein domain, the phenotype/s of the patients, the gene dosage, and the experimental evidence are indicated. Het: heterozygosity, Homo: homozygosity; Het*: compound heterozygosity, FF: fertilization failure, FR: fertilization rate. The colour code provides information on the pathogenicity for each variant: no experimental validation reported (grey), reported as benign (green), reported as pathogenic (orange), potentially pathogenic or unclear according to existing data (yellow)

Affected gene	Variant (protein)	Variant (cDNA)	Gene location	Affected protein domain	Mutation type	dbSNP ID	Dose	Experimental validation	No. patients	Reference/s	Phenotype/s
PATL2	p.R75Vfs⊠21	c.223−14_223−2del	Exon 3	–	Splicing	–	Het*	*PATL2* cDNA sequencing – Pathogenic	1+3	Wu *et al.*, 2019	Oocyte maturation arrest, FF and embryo arrest
	p.L189R	c.566T>G	Exon 5	–	Missense	–	Het*	*In silico*, WB analysis of protein levels in transfected cells – Potentially pathogenic	1	Chen *et al.*, 2017b	FF, zygotic arrest
	p.Y217N	c.649T>A	Exon 5	–	Missense	–	Het*	*In silico*, WB analysis of protein levels in transfected cells – Potentially pathogenic	1	Chen *et al.*, 2017b	FF, zygotic arrest
	p.N239fs*9	c.716delA	Exon 6	–	Frameshift deletion	–	Het*	No	1	Wu *et al.*, 2019	FF
	p.R280Q	c.839G>A	Exon 7	PAT1	Missense	–	Het*	*In silico*, WB analysis of protein levels in transfected cells – Unclear	1	Chen *et al.*, 2017b	FF, zygotic arrest
	p.I318T	c.953T>C	Exon 8	PAT1	Missense	–	Het*	*In silico*, WB analysis of protein levels in transfected cells – Pathogenic	1	Chen *et al.*, 2017b	FF, zygotic arrest
TLE6	p.R409Q	c.1226G>A	Exon 13	WD40 domain repeats	Missense	rs1310808966	Homo	*In silico* – Unclear	1	Lin *et al.*, 2020	FF
	p.S510Y	c.1529C>A	Exon 15	WD40 domain repeats	Missense	rs767222404	Homo	*In silico*, immunoblot analyzing TLE6 phosphorylation levels and dephosphorylation assay, *in vitro* analysis of PKA phosphorylation, analysis of the interaction between mutated TLE6 and other SCMC components by IP – Pathogenic	2	Alazami *et al.*, 2015	FF
	p.E541K	c.1621G>A	Exon 17	WD40 domain repeats	Missense	rs1176718387	Homo	*In silico* – Potentially pathogenic	1	Lin *et al.*, 2020	Low FR
PADI6	p.R457*	c.1369C>T	Exon 11	Protein arginine deiminase (PAD)	Nonsense, stop gain	rs766389713	Homo	No	1	Maddirevula *et al.*, 2017	Zygotic arrest
	p.S508Qfs*5	c.1521dupC	Exon 13	Protein arginine deiminase (PAD)	Frameshift insertion	–	Homo	No	1	Zheng *et al.*, 2020a	Low FR, abnormal fertilization
TRIP13	p.I198V	c.592A>G	Exon 6	AAA+ ATPase domain	Missense	–	Het*	*In silico*, analysis of protein levels and HORMAD2 levels in transfected cells – Unclear	1	Zhang *et al.*, 2020	Zygotic arrest
	p.V247M	c.739G>A	Exon 8	AAA+ ATPase domain	Missense	rs1203102465	Het*	*In silico*, analysis of protein levels and HORMAD2 levels in transfected cells – Pathogenic	1	Zhang *et al.*, 2020	Zygotic arrest

(*Continued*)

Table 5. (*Continued*)

Affected gene	Variant (protein)	Variant (cDNA)	Gene location	Affected protein domain	Mutation type	dbSNP ID	Dose	Experimental validation	No. patients	Reference/s	Phenotype/s
BTG4	p.M1?	c.1A>G	Exon 2	BTG domain	Missense, start lost	rs755402000	Homo	*In silico*, analysis of protein levels in transfected cells – Pathogenic	1	Zheng *et al.*, 2020b	Zygotic arrest
	p.Q25*	c.73C>T	Exon 2	BTG domain	Nonsense, stop gain	–	Homo	*In silico*, analysis of protein levels in transfected cells – Pathogenic	1	Zheng *et al.*, 2020b	Zygotic arrest
	p.A56T	c.166G>A	Exon 2	BTG domain	Missense	–	Homo	*In silico*, analysis of protein levels in transfected cells, analysis of BGT4–CNOT7 interaction by co-IP – Pathogenic	1	Zheng *et al.*, 2020b	Zygotic arrest
	p.W95C	c.285G>C	Exon 3	BTG domain	Missense	–	Homo	Analysis of BGT4–CNOT7 interaction by co-IP – Pathogenic	1	Liu *et al.*, 2021	Zygotic arrest
	p.I159Lfs*15	c.475_478del	Exon 4	–	Frameshift deletion	–	Homo	*In silico*, analysis of protein levels in transfected cells – Pathogenic	1	Zheng *et al.*, 2020b	Zygotic arrest
ZAR1	p.P172=	c.516C>T	Exon 1	–	Synonymous	rs755756974	Het	*In silico* – Benign	1	Tian *et al.*, 2020	Zygotic arrest
NLRP5	p.R143P	c.428G>C	Exon 2	Pyrin	Missense	rs746147069	Het*	*In silico* – Potentially pathogenic	1	Zheng *et al.*, 2021b	FF
	p.R462C	c.1384C>T	Exon 6	NACHT	Missense	rs199475775	Het*	*In silico* – Unclear	1	Zheng *et al.*, 2021b	FF
	p.R533P	c.1598G>C	Exon 6	NACHT	Missense	rs752560793	Het*	*In silico* – Potentially pathogenic	1	Li *et al.*, 2021	FF
	p.L640R	c.1919T>G	Exon 6	–	Missense	–	Het*	*In silico* – Potentially pathogenic	1	Li *et al.*, 2021	FF
	p.W759Dfs*4	c.2274_2275del	Exon 7	LRR	Frameshift deletion	–	Homo	*In silico* – Potentially pathogenic	1	Maddirevula *et al.*, 2020	FF
	p.S893T	c.2677T>A	Exon 10	LRR	Missense	rs769920247	Het*	*In silico* – Unclear	1	Zheng *et al.*, 2021b	FF
	p.L1116W	c.3347T>G	Exon 14	LRR	Missense	rs773877703	Het*	*In silico* – Unclear	1	Zheng *et al.*, 2021b	FF
NLRP7	p.K619Nfs*6	c.1857_1858del	Exon 4	LRR	Frameshift deletion	rs749049980	Homo	*In silico* – Potentially pathogenic	1	Maddirevula *et al.*, 2020	FF, abnormal fertilization
CDC20	p.Y228C	c.683A>G	Exon 6	WD40 repeats	Missense	rs1309457938	Homo	*In silico* – Potentially pathogenic	1	Xu *et al.*, 2021	Oocyte maturation arrest, FF
	p.R322*	c.964C>T	Exon 7	WD40 repeats	Nonsense, stop gain	–	Het*	*In silico,* Western blot in CHO cells transfected, function rescue in *Cdc20* knockdown mouse oocytes – Pathogenic	1	Zhao *et al.*, 2021	FF
	p.R322Q	c.965G>A	Exon 7	WD40 repeats	Missense	–	Het*	*In silico,* western blot in CHO cells transfected, function rescue in *Cdc20* knockdown mouse oocytes – Pathogenic	1	Zhao *et al.*, 2021	FF

observed in the infertile women previously mentioned. Indeed, these variants cause a reduction in CDC20 protein levels and, for the p.R322* variant, the generation of a non-functional truncated protein (Zhao *et al.*, 2021). All three *CDC20* variants reported in this review directly affect the WD40 repeats, a region required for protein–protein interactions (Figure 3; Kraft *et al.*, 2005).

Another affected gene is *PATL2*, with six variants identified in three women, with significant phenotypic variability: oocyte maturation arrest, FF, low FR, cleavage failure, and embryo developmental arrest (Chen *et al.*, 2017a). *PATL2* encodes for PAT1 homolog 2 (PATL2), an mRNA-binding protein that, in the oocyte, regulates the expression of specific mRNAs encoding proteins essential for oocyte maturation, meiotic progression and early embryo development (Nakamura *et al.*, 2010). Most *PATL2* exonic variants reported to date are pathogenic or potentially pathogenic (Table 5).

Similarly to *TUBB8* variants, the phenotypic variability observed for PATL2 variants may imply a difficult diagnosis, as some result in complete maturation arrest (in some cases, already at the GV stage), while others are associated with a high percentage of apparently normal MII oocytes, which stop their development a few hours after insemination.

PATL2 variants seem to affect protein function or reduce the gene's expression to different extents both in oocytes and in transfected cells, therefore resulting in different phenotypes, mainly oocyte maturation arrest (either at the GV or MI stage; Wu *et al.*, 2019). For example, ~26–44% of patients with oocyte maturation arrested at the GV stage carry *PATL2* pathogenic variants (Christou-Kent *et al.*, 2018; Huang *et al.*, 2018). However, there is no evidence that the reported *PATL2* variants have affected PATL2 function as an mRNA-binding protein and its effect on mRNA translation in the oocyte, perhaps because the function of PATL2 is still largely unknown. For one splice variant identified in this gene (p.R75Vfs*21), sequencing of cDNA obtained from granulosa cells in the affected individuals showed the generation of abnormal transcripts, leading to truncated non-functional forms of the PATL2 protein (Chen *et al.*, 2017a).

Recently, bi-allelic pathogenic variants in *TRIP13* have been identified in an infertile woman (Table 5); this woman had normal fertilization, but the zygotes failed to undergo the first mitotic division (Zhang *et al.*, 2020). *TRIP13* encodes for thyroid hormone receptor interactor 13 (or Pachytene checkpoint protein 2 homolog), a factor with ATPase activity involved in meiotic recombination and essential for chromosome synapsis (Roig *et al.*, 2010).

Apart from p.I198V and p.V247M (included in the present review), other *TRIP13* variants have been reported in infertile women who could produce a variable proportion of morphologically normal MII oocytes. Among the molecular alterations caused by *TRIP13* variants there is a reduction of TRIP13 protein abundance, an inability to remove HORMAD2 from synapse chromosome axes (a checkpoint required for meiotic progression), and reduced ATPase activity (Zhang *et al.*, 2020). Indeed, both missense variants reported by Zhang and colleagues directly affected the AAA+ ATPase domain (Figure 3).

The four next genes implicated in FF and zygotic arrest are part of the subcortical maternal complex (SCMC). The SCMC is a complex of proteins encoded by maternal-effect genes that controls the first cellular divisions of the preimplantation embryo (Bebbere *et al.*, 2016). SCMC orchestrates the zygotic events occurring in parallel to oocyte activation, including the regulation of subcortical actin and mitotic spindle positioning, the regulation of the translation of maternal transcripts and the epigenetic reprogramming (Li *et al.*, 2008; Bebbere *et al.*, 2016). Functional studies using the mouse model have shown that the knockout for these factors causes lethal phenotypes such as preimplantation embryo arrests at the cleavage stage and, in some cases, at the zygotic stage (Tashiro *et al.*, 2010). The first is *TLE6*, which encodes for transducin-like enhancer protein 6 (TLE6); three variants affecting four women with FF after ICSI have been reported (Table 5). The crucial role of SCMC is demonstrated by the phenotypes of the patients carrying the TLE6 p.S510Y variant in homozygosity, who present a dramatic phenotype: three oocytes developing to 2PN among 77 inseminated by ICSI from a total of seven cycles (3.9% FR; Alazami *et al.*, 2015). The molecular mechanisms behind this phenotype are explained by some authors: specific *TLE6* variants can disturb TLE6 protein phosphorylation sites (as demonstrated *in vitro* in transfected cells) and compromise the ability of the protein to be phosphorylated by PKA, as well as inhibit its interaction with other SCMC components such as OOEP and KDHC3L, altogether preventing the meiotic resumption of the inseminated oocyte (Alazami *et al.*, 2015).

The second gene is *PADI6*, which encodes for protein arginine deiminase type-6 (PADI6); with two variants identified in two infertile women (Table 5). Its knockout in mice (Padi6$^{-/-}$) caused embryo developmental arrest mainly at the 2-cell stage, the stage in which mouse embryonic genome activation (EGA) occurs (Yurttas *et al.*, 2008). In humans, three waves of transcriptional genome activation occur at the 2-cell, 4-cell and 6- to 10-cell stages (Vassena *et al.*, 2011) and, consistently, Xu and colleagues identified PADI6 variants in infertile women presenting embryonic arrest at these stages (Xu *et al.*, 2016). In these women, the oocytes lacked detectable PADI6 protein in the cytoplasm, and arrested embryos had reduced levels of phosphorylated RNA polymerase II, something compromising EGA (Xu *et al.*, 2016). As indicated in Table 5, in some cases all these alterations can clinically manifest already at the zygotic stage, causing poor FR, abnormal fertilization or zygotic arrest (Maddirevula *et al.*, 2017; Zheng *et al.*, 2020a).

The third gene is *NLRP5* (NLR Family Pyrin Domain Containing 5, also known as MATER). While some studies have reported genetic variants in infertile patients presenting embryonic arrest (Mu *et al.*, 2020; Xu *et al.*, 2020), seven variants have been identified in four women experiencing FF after ICSI (Table 5). Similarly, one mutation in a fourth SCMC factor – *NLRP7* (NOD-like receptor family pyrin domain containing 7) – has been recently identified in a woman with FF (Maddirevula *et al.*, 2020). Other components of the SCMC are *KHDC3L*, *OOEP*, *ZBED3* and *NLRP2*, but genetic variants previously reported in these genes were associated exclusively with embryo arrest at the cleavage stage, without any apparent effect at the zygote stage (Zheng *et al.*, 2021b).

For *NLRP5* and *NLRP7* variants, only *in silico* approaches have been performed to predict their effects, so further research is required to gain insight into their specific molecular pathogenesis. However, both proteins have an N-terminal pyrin domain required for binding adaptor proteins, a central nucleotide-binding oligomerization domain (NACHT) and a C-terminal leucine-reach repeat (LRR) for ligand binding and the establishment of protein–protein interactions that regulate cellular functions (Proell *et al.*, 2008). As shown in Figure 3, most variants detected in infertile patients affect these domains. In mouse oocytes and embryos, NLRP5 or MATER was involved in mitochondrial activation, endoplasmic reticulum distribution and calcium homeostasis (Fernandes *et al.*, 2012; Kim *et al.*, 2014). For this reason,

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

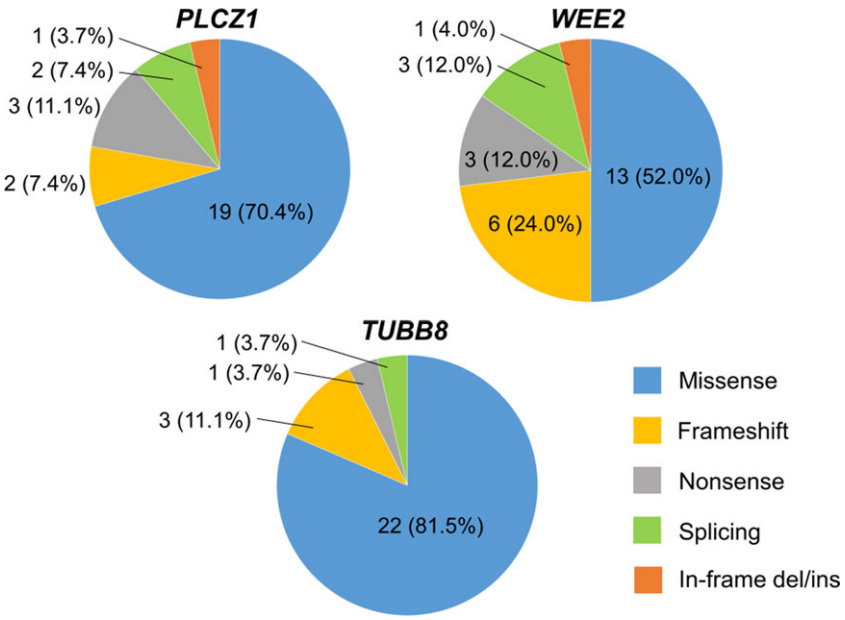

Figure 4. Proportion of each type of genetic variant for the three genes in which more genetic variants have been identified in infertile patients presenting FF or zygotic arrest after ICSI: *PLCZ1*, *WEE2* and *TUBB8*.

pathogenic variants in these SCMC genes could impair protein structure and/or function, altering cellular functions during the zygote stage.

Regarding SCMC, genetic variants affecting *PADI6*, *NLRP5* and *NLRP7* (and other SCMC genes such as *KHDC3L*) have been associated with the occurrence of hydatidiform moles, which are aberrant pregnancies characterized by the absence of, or abnormal, embryonic development, and hyperproliferation of the trophoblast or placenta (Qian *et al.*, 2018; Fallahi *et al.*, 2020). The role of these genes in setting the genomic imprinting could be an explanation (Docherty *et al.*, 2015). Interestingly, the occurrence of hydatidiform moles can be male related also, associated with sperm unable to trigger the normal pattern of Ca^{2+} oscillations and/or the presence of pathogenic variants in *PLCZ1* (Nikiforaki *et al.*, 2014; Guggilla *et al.*, 2019). Altogether, these observations not only increase the spectrum of clinical consequences associated with alterations in factors involved in early fertilization, but also confirm that calcium oscillations and early fertilization events affect the future development of the embryo.

Finally, *BTG4* and *ZAR1* are two genes associated with female-related zygotic arrest. Five *BTG4* exonic variants were identified in homozygosity in five patients, all of them presenting morphologically normal oocytes that could be successfully fertilized by ICSI (2PN formed), but embryo development was arrested at the zygotic stage (Zheng *et al.*, 2020b). *BTG4* (B cell translocation gene 4) is a key factor for maternal–zygotic transition (MZT). BTG4 forms a complex with CNOT7 and EIF4E, facilitating the decay of maternal mRNAs during the zygotic stage, an oocyte cytoplasmic maturation step required for MZT and the first mitotic division to occur (Yu *et al.*, 2016). The mechanisms by which BTG4 variants produce zygotic arrest are the reduction or absence of BTG4 (for c.1A>G and p.Q25* variants), generation of truncated non-functional protein (for p.I159Lfs*15), or an altered BGT4–CNOT7 interaction (for the p.A56T variant), problems causing a loss of maternal mRNA decay in the zygote, abnormal MZT and the subsequent cleavage failure (Zheng *et al.*, 2020b). Three out of four variants reported are located at the N-terminal region of the protein, directly affecting the BGT domain (Figure 3).

ZAR1 encodes Zygote arrest protein 1, and studies using the murine model have demonstrated its essential role in early fertilization: $Zar1^{-/-}$ female mice were infertile, and less than 20% of fertilized zygotes underwent the first mitotic division (Wu *et al.*, 2003). A recent study analyzed the *ZAR1* sequence in 47 patients who presented cleavage failure after ICSI and in 188 fertile women, and one exonic variant was exclusively identified in the first group (Table 5). However, the synonymous variant in *ZAR1* (c.516C>T) was not a cause of infertility, probably because there was no change in the protein sequence and variants in simple heterozygosity are not expected to cause a phenotype of zygotic arrest ($Zar^{+/-}$ female mice are fertile) (Wu *et al.*, 2003). Future studies may be helpful to clarify if *ZAR1* variants in homozygosity can cause alterations in oocyte-to-embryo transition in humans.

Nature and inheritance of genetic variants associated with FF and zygotic arrest

Most variants compiled in the present review are missense ($n = 82$; 67.8%), and the rest of the variants were frameshift (either insertion or deletions), nonsense or stop gain, splicing variants, in-frame deletions or insertions, whereas one patient carried a synonymous variant. For the three genes with a higher number of different variants identified in infertile patients (*PLCZ1*, *WEE2*, and *TUBB8*), the proportion of each type of variant is indicated in Figure 4. Pathogenic effects are expected especially for frameshift, nonsense, and splicing variants, as they mostly result in truncated or aberrant proteins. However, most missense variants reported here also generate dramatic effects on protein structure and/or function, characterized by diminished or lost enzymatic function, altered ability to interact with the substrate or protein partners, alteration of phosphorylation sites, or reduction of expression levels.

Among the 141 infertile men and women included in this review, 46 (32.6%) carried a single variant in heterozygosity, 89 carried bi-allelic variants, either in homozygosity ($n = 55$; 39.0%) or in compound heterozygosity ($n = 34$; 24.1%); while the inheritance pattern was unknown for six patients (4.3%).

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

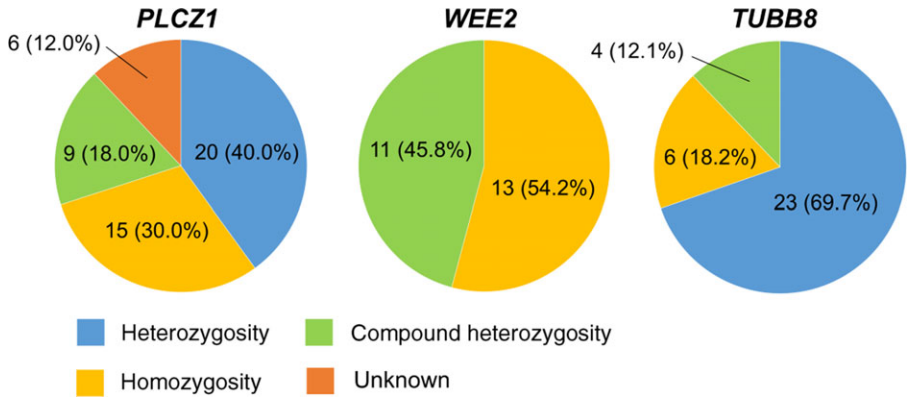

Figure 5. Proportion of patients presenting genetic variants in homozygosity, heterozygosity or compound heterozygosity for the genes affecting a higher number of infertile patients: *PLCZ1*, *WEE2* and *TUBB8*.

However, while pathogenic variants in heterozygosity have been identified in infertile patients for *PLCZ1* and *TUBB8*, other genes (*WEE2*, *PATL2*, *TLE6*, *PADI6*, *BTG4, NLRP5, NLRP7*, *CDC20*, *TRIP13*, *DNAH17*, *ACTL9* and *ACTLA7*) seem to cause a phenotype of infertility in a recessive manner, as both alleles are mutated in all patients identified. Moreover, for this second group of genes, many studies included the relatives of the affected patients in the analysis, demonstrating that variants in heterozygosity did not affect fertility and overall health (Alazami *et al.*, 2015; Chen *et al.*, 2017b; Sang *et al.*, 2018; Zhang *et al.*, 2020; Zheng *et al.*, 2020b). In Figure 5, the distribution of the genetic load of the variants is represented for the three genes affecting a higher number of patients (*PLCZ1*, *WEE2* and *TUBB8*).

After the first reports using gene sequencing to study male-related FF, the initial hypothesis was that pathogenic *PLCZ1* variants needed to be present in both maternal and paternal alleles to cause infertility (Kashir *et al.*, 2012). However, nearly half the men with FF or low FR included in this study carried *PLCZ1* in simple heterozygosity (20/46, 43.5%; Figure 5), suggesting that heterozygosity may be sufficient to cause oocyte activation deficiencies for certain variants. If this is the case, some *PLCZ1* variants would be acting in a dominant negative mechanism; while this statement needs further research and confirmation, there are some facts that support it. For instance, spermatids share cytoplasmic bridges during their development, interchanging RNA transcripts, implicating that variants in heterozygosity could cause a population of testicular sperm genetically different (part of the sperm would be wild-type) but phenotypically similar (Dadoune *et al.*, 2004). Indeed, none of the pathogenic or potentially pathogenic *PLCZ1* variants indicated in Table 1 has been found in patients or donors with good FR, commonly used as controls in different studies. Second, the presence of mutated forms of PLCζ could affect the ability of the wild-type protein to trigger calcium oscillations in the oocyte, as demonstrated by co-injecting wild-type and mutant forms of human recombinant rLCζ into mice oocytes (Heytens *et al.*, 2009). Third, especially for genetic variants causing a total disruption of PLCζ enzymatic activity (such as p.V189Cfs*12, p.C196* and p.V326Kfs*25), heterozygosity would either reduce the amount of wild-type protein present in all sperm or at least reduce the percentage of sperm with wild-type protein among the whole sperm population, diminishing the chances to achieve oocyte activation after ICSI, especially in oocytes with cytoplasmic immaturity. The same is true for variants in domains like C2, which may prevent PLCζ protein from reaching and attaching to the perinuclear theca, being disposed through the residual body and accumulating in abnormal areas within the oocyte after fertilization (Escoffier *et al.*, 2016). The complexity of human fertilization, and the different *PLCZ1* variants can explain why the phenotype observed in the patients ranges from complete infertility to subfertility or low FR, and possibly explain why the fathers of the patients included, carrying *PLCZ1* variants in heterozygosity, were able to generate offspring *in vivo* (Torra-Massana *et al.*, 2019; Yan *et al.*, 2020). This phenotypical behaviour, which may seem contradictory, is similar in *Plcz1* KO mice: while total FF with a complete absence of calcium oscillations is observed through ICSI, offspring could be produced *in vivo* (Nozawa *et al.*, 2018; Hachem *et al.*, 2017).

Regarding *TUBB8*, the same phenotypes are observed for both monoallelic and bi-allelic variants (Figure 5). As previously reported, *TUBB8* variants can have dominant negative effects on microtubule function (Feng *et al.*, 2016). Indeed, in affected infertile women, most *TUBB8* variants are either *de novo* or inherited from their fathers (alterations in TUBB8 do not cause male infertility; Feng *et al.*, 2016). Surprisingly, in some cases the variants were inherited from the mother, which could be explained by differences in the protein structure abnormalities caused by different variants, or incomplete penetrance (Chen *et al.*, 2017b, 2019).

In contrast, all *WEE2* variants identified were either in homozygosity or compound heterozygosity (Figure 5). The inheritance of *WEE2* pathogenic variants is autosomal recessive, and carriers of just one mutated allele do not display infertility (Zhao *et al.*, 2019). This inheritance pattern is shared by maternal-effect genes associated with the SCMC (*TLE6*, *NLRP5*, *NLRP7* and *PADI6*) and by the rest of the genes compiled in the present review, either associated with female infertility (*PATL2*, *TRIP13* and *BGT4*) or male infertility (*ACTL9, ACTLA7* and *DNAH17*). For all genes with recessive inheritance, the proportion of infertile individuals is expected to be higher in families with a history of consanguinity when compared with the general population. In fact, among the 54 infertile patients carrying homozygous variants in the studies included in the present review, at least 29 (53.7%) were members of consanguineous families. For this reason, the possibility of consanguinity and the genetic information of the closest relatives should be considered when addressing the genetic analysis of male or female infertility associated with FF or zygotic arrest.

Treatment options and genetic counselling for patients carrying variants associated with FF and zygotic arrest

The treatment options for patients with FF or zygotic arrest are still scarce but, as indicated in Table 6, some experimental alternatives have been reported recently for patients affected by *PLCZ1*, *WEE2*, *ACTL9, ACTLA7* and, to a lesser extent, *TUBB8*, *CDC20* and

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Table 6. Summary of treatment options for patients carrying infertility-related genetic variants causing fertilization failure or zygotic arrest after ICSI. The experimental status of these treatments and its clinical efficiency are also indicated.

Gene	Phenotype/s	Treatment to overcome/ palliate the infertility problem	Status	Tested on affected patients	Reference/s
PLCZ1	FF	AOA using calcium ionophores	Clinical application	Yes	Torra-Massana *et al.*, 2019; Dai *et al.*, 2021; Mu *et al.*, 2020; Yan *et al.*, 2020; Wang *et al.*, 2020
		Injection of *PLCZ1* cRNA	Experimental	No	Yamaguchi *et al.*, 2017
WEE2	FF	AOA using calcium ionophores	Invalid treatment choice	Yes	Yang *et al.*, 2019; Dai *et al.*, 2019; Wang *et al.*, 2021
		Injection of *WEE2* cRNA	Experimental	Yes	Sang *et al.*, 2018
TUBB8	Oocyte maturation arrest, FF, low FR, zygotic arrest	AOA using calcium ionophores	Experimental	No	Yuan *et al.*, 2018
CDC20	Oocyte maturation arrest, FF, low FR	Injection of *CDC20* cRNA	Experimental	No	Zhao *et al.*, 2021
TRIP13	FF, zygotic arrest	Injection of *TRIP13* cRNA	Experimental	Yes	Zhang *et al.*, 2020
NLRP5	FF	AOA	Invalid treatment choice	Yes	Li *et al.*, 2021
DNAH17	FF	AOA	Invalid treatment choice	Yes	Jia *et al.*, 2021
ACTL9	FF	AOA using calcium ionophores	Experimental	Yes	Dai *et al.*, 2021
ACTL7A	FF	AOA using calcium ionophores	Experimental	Yes	Wang *et al.*, 2021

TRIP13 pathogenic variants. In contrast, no treatment options have been attempted or proposed for patients with *DNAH17*, *PATL2*, *TLE6*, *PADI6*, *NLRP5, NLRP7* and *BTG4* variants, while variants identified in *WBP2NL* and *ZAR1* are considered non-pathogenic.

Fortunately, ICSI combined with AOA using calcium ionophores has proven to be effective for infertile men carrying deleterious *PLCZ1* variants (Torra-Massana *et al.*, 2019; Yan *et al.*, 2020), and AOA is also effective in cases of *ACTL9* and *ACTLA7* variants to overcome the FF problem (Dai *et al.*, 2021). The use of AOA generates an increase in the oocyte cytoplasmic levels of calcium. Apparently, this signal is enough to compensate for the sperm's inability to do so and initiate embryo development. For this reason, genetic screening of at least *PLCZ1* should be encouraged in cases of OAF after ICSI of suspected male origin, and used for supporting the use of AOA in future ICSI cycles. Recently, Cheung *et al.* demonstrated the necessity of confirming sperm-related OAD before exposure of the gametes to the chemicals used in AOA (Cheung *et al.*, 2020). Alternatively, the injection of *PLCZ1* wild-type cRNA or recombinant PLCZ1 protein is demonstrated to trigger oocyte activation in mature oocytes, and some studies have already set the protocol conditions to use this method in humans (Yoon *et al.*, 2012; Nomikos *et al.*, 2013; Yamaguchi *et al.*, 2017). This method has been proposed as a better option compared with AOA, as the calcium oscillations generated are much more similar to the outcomes in an *in vivo* situation. Nevertheless, no evidence of a benefit in the rPLCζ–AOA protocol over the one using the Ca^{2+} ionophore was observed when considering preimplantation embryo development (Ferrer-Buitrago *et al.*, 2020).

Conversely, the FF problem originated by *WEE2* and *NLRP5* variants (in women) or DNAH17 alterations (in men) is not overcome by AOA using calcium ionophores (Table 6). AOA seems to enable the extrusion of the second polar body in oocytes with pathogenic *WEE2* variants inseminated by ICSI, but these oocytes failed to produce pronuclei (Sang *et al.*, 2018; Yang *et al.*, 2019). As suggested by other authors, the presence of non-functional WEE2 led to high levels of MPF that prevented the oocyte from completing meiotic resumption, and AOA will not benefit these patients. A correct diagnosis before treatment counselling gains relevance in this scenario, as previous studies have reported that AOA did not overcome the FF problem in some patients, especially in cases of suspected oocyte-related origin (Combelles *et al.*, 2010; Vanden Meerschaut *et al.*, 2012; Ferrer-Buitrago *et al.*, 2018). Recent evidence has indicated that injection of *WEE2*, *TRIP13* or *CDC20* wild-type cRNA may be a viable alternative for infertile women carrying pathogenic variants, but further research needs to be conducted to test the safety and efficiency in human oocytes and to evaluate any potential damaging effects on the developing embryo (Yang *et al.*, 2019; Table 6).

While AOA may be a good option for part of the cases of OAF, this is not so clear for patients successfully forming 2PN but failing to undergo the first mitotic division. As previously detailed, arrest at the pronuclear stage or cleavage failure is a phenotype observed in some patients carrying variants in *TLE6*, *TUBB8* or *PADI6*, or *BTG4*. Preliminary studies have shown that AOA could be a good option for some patients presenting this phenotype (Ebner *et al.*, 2015), but further research is required to confirm this hypothesis (Darwish and Magdi, 2015). Interestingly, a recent study in the mouse has demonstrated that the genome transfer technique could

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

be useful in overcoming the infertility condition originated by mutations in maternal-effect genes (Bai *et al*., 2020) but, currently, its application is just speculative. Oocyte spindle transfer could be another potential option for patients showing oocyte-related FF or embryo arrest (Costa-Borges *et al*., 2020). This technique was used for the first time in humans in 2016 to prevent the transmission of Leigh syndrome (a mitochondrial disease), and led to the birth of a healthy boy (Zhang *et al*., 2017).

Despite the dramatic clinical consequences caused by the variants described in this review, patients with partial FF or zygotic arrest as well as patients in whom the infertility problem can be overcome by an alternative treatment can produce viable embryos and transmit the variants to the offspring. This risk is higher for variants presenting a dominant inheritance, when a single affected allele causes a phenotype of infertility. In these patients, preimplantation genetic testing (PGT-M) could be an option to select embryos not affected by the variants, when allowed. However, so far there has been no evidence supporting the clinical utility or appropriateness of PGT-M for genetic alterations that do not cause severe diseases or affect the general quality of life.

For all of these reasons, genetic counselling should be offered to patients carrying variants causing FF, cleavage failure or other infertility problems. These patients should be informed about the diagnostic value of genetic screening and the potential risks of transmission to the offspring, but should be offered the possibility to evaluate the family history, coordinate the specific tests needed to investigate a genetic cause for infertility and receive professional support to cope with the emotional consequences of repeat failure to achieve a pregnancy. Unfortunately, only four out of 45 (8.9%) studies included in the present review mention the need for genetic counselling for these infertile patients. As the knowledge of the genetic basis of human infertility expands, genetic counselling practice is expected to do so.

Discussion

The results of the present review demonstrated that high numbers of genetic alterations are associated with FF and zygotic arrest after ICSI, and that most of them are pathogenic (except for *WBP2NL* and *ZAR1* genes). The high effect of these alterations on the fertilization process is exemplified by the search for WEE2 inhibitors to be used as a non-hormonal contraceptive strategy (Hanna *et al*., 2019).

Lower fertilization rates or oocyte activation deficiencies are somehow expected for patients with severe sperm parameters (such as severe asthenozoospermia, cryptozoospermia or use of testicular sperm) or some oocytes abnormalities, such as the presence of cytoplasmic vacuoles (Ebner *et al*., 2005). For this reason, we only focused on patients presenting morphologically normal sperm and at least some morphologically normal and mature oocytes, as these cases are often unexpected. Nevertheless, previous studies have identified specific genetic variants associated with abnormal sperm morphology and, as a consequence, compromised fertilization ability after ICSI, including mutations in *DPY19L2* and *SPATA16* that cause globozoospermia (Rybouchkin *et al*., 1996; Dam *et al*., 2007).

Different approaches have been tested to study gametes from patients with FF through ICSI, for example the analysis of PLCZ1 protein levels and subcellular localization, as well as diverse functional assays. For example, heterologous ICSI models, using mouse or hamster oocytes in most cases, have been proposed as a diagnostic tool for sperm-related FF (Yazawa *et al*., 2000; Heindryckx *et al*., 2005). These systems include the mouse oocyte activation test (MOAT) and mouse oocyte calcium analysis (MOCA; Vanden Meerschaut *et al*., 2013); as well as its variant in human gametes, HOCA (Ferrer-Buitrago *et al*., 2018). Similarly, heterologous models using bovine, rabbit or *Xenopus* oocytes have been applied to test sperm aster formation and/or microtubule enucleation (Tachibana *et al*., 2009; Amargant *et al*., 2018). However, these methods cannot easily be introduced in routine fertility clinical practice due to technical or cost–effectiveness limitations. In contrast, gene sequencing does not provide functional insights but offers distinct advantages: it is fast, relatively cheap, and does not require complex protocols or reagents when provided by an external service; it is easy to implement in fertility clinics or associated centres, it can also be applied to the patient's parents or other relatives to analyze the inheritance profile and the origin of each specific variant, and it can be performed on a very small amount of sample (even a single cell) or using samples other than gametes (such as blood or saliva), especially useful as mature oocytes or semen presenting severe oligozoospermia are limiting samples.

The complexity of fertilization, the high diversity of male and female factors involved, as well as the ethical, legal and technical limitations to investigate this process in humans explain why fertilization and its alterations are still not completely understood. Nevertheless, many genes can be considered potential diagnostic targets. In men, genetic alterations causing poor sperm DNA condensation or protamine deficiency, sperm proteasome function, abnormal sperm head-neck junction and altered sperm centriole structure or function may explain the additional cases of male-related FF or zygotic arrest after ICSI (Chemes *et al*., 1999; Rawe *et al*., 2002, 2008; Lazaros *et al*., 2011). Potential targets in sperm could be *TSSK6*, involved in histone-to-protamine transition during spermiogenesis (Jha *et al*., 2017), or *GSTO2*, encoding an enzyme located in the sperm post-acrosomal sheath that, if inhibited, causes a delay in sperm decondensation and lower rates of zygotic division (Hamilton *et al*., 2019).

In women, the potential targets to identify infertility-related genetic variants are diverse. Apart from *TUBB8*, genetic variants in factors involved in oocyte spindle structure and function and microtubule nucleation such as PLK1 and DCTN3 could cause failed ICSI outcomes (Fan *et al*., 2015). Similarly, novel pathogenic variants could be found in oocyte factors involved in sperm decondensation, such as HIRA and the recently described splicing kinase SRPK1 (Gou *et al*., 2020), but none has been identified in humans so far. As exemplified by *PATL2*, genetic alterations in other genes affecting oocyte maturation can cause an inability of MII oocytes to undergo complete activation. Similarly, as found with *WEE2* and *CDC20*, genetic alterations affecting additional factors involved in the oocyte activation signalling pathway could explain part of the FF and zygotic arrest after ICSI. For example, oocytes from mice knockout for *Smc1b* and *Mos* are arrested at meiosis II (Araki *et al*., 1996; Revenkova *et al*., 2004). In humans, some genetic variants in *MOS* have been recently identified in infertile women experiencing preimplantation embryonic arrest and embryo fragmentation, but none has been associated with OAF or with arrest prior to the first mitotic division (Zhang *et al*., 2021; Zeng *et al*., 2022).

Some limitations may exist in the present review. For example, there is a risk of incomplete retrieval of published articles matching the research question, a risk that was reduced by evaluating different sources and performing a manual analysis of references for each study identified. In addition, due to the multiplicity of phenotypes originating from different variants in the same gene, it

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

is possible that the present catalogue may have skipped some variants described in patients presenting alternative phenotypes (such as complete oocyte maturation arrest or preimplantation developmental arrest at the cleavage stage) but with the potential to cause FF or zygotic arrest. Finally, as may happen in other similar reviews, not all ethnicities are represented, as most studies include patients of Asian origin. Future research is needed to overcome this limitation, by providing an independent verification on additional families and ethnic groups.

In summary, according to the existing evidence, 14 out of 16 genes included in the present review are potential targets for genetic screening of variants related to FF or zygotic arrest after ICSI: *PLCZ1*, *DNAH17*, *ACTL9* and *ACTLA7* (in infertile men), and *WEE2*, *TUBB8*, *PATL2*, *TLE6*, *PADI6*, *TRIP13*, *BTG4*, *CDC20, NLRP5*, and *NLRP7* (in infertile women). Pathogenic variants in some of these genes (such as *PLCZ1* and *WEE2*) can explain a large proportion of unexpected and often repetitive FF cases. Many more targets and novel variants are expected to appear in the near future not only related to FF, but also to all types of infertility, allowing the application of what is known as genome-based reproductive medicine or preconception genomic medicine (Capalbo *et al.*, 2021).

Supplementary material. For supplementary material accompanying this paper visit https://doi.org/10.1017/S096719942300014X

Financial support. This work was supported by intramural funding from Eugin.

Competing interest. The authors report no financial or commercial conflicts of interest.

References

Aarabi, M., Balakier, H., Bashar, S., Moskovtsev, S. I., Sutovsky, P., Librach, C. L. and Oko, R. (2014). Sperm-derived WW domain-binding protein, PAWP, elicits calcium oscillations and oocyte activation in humans and mice. *FASEB Journal*, **28**(10), 4434–4440. doi: 10.1096/fj.14-256495

Alazami, A. M., Awad, S. M., Coskun, S., Al-Hassan, S., Hijazi, H., Abdulwahab, F. M., Poizat, C. and Alkuraya, F. S. (2015). TLE6 mutation causes the earliest known human embryonic lethality. *Genome Biology*, **16**, 240. doi: 10.1186/s13059-015-0792-0

Amargant, F., García, D., Barragán, M., Vassena, R. and Vernos, I. (2018). Functional analysis of human pathological semen samples in an oocyte cytoplasmic ex vivo system. *Scientific Reports*, **8**(1), 15348. doi: 10.1038/s41598-018-33468-x

Amdani, S. N., Jones, C., Späth, K., Babariya, D., Malinauskas, T., Gemmell, L., Ferrer-Buitrago, M., Heindryckx, B., Wells, D., De Sutter, P. and Coward, K. (2016). P-095 Next generation sequencing reveals a novel mutation in the XY-linker region of phospholipase C zeta (PLCζ), resulting in truncated protein and oocyte activation deficiency. *Human Reproduction*, **31**(Suppl_1), i170. doi: 10.1093/humrep/31.Supplement_1.1

Araki, K., Naito, K., Haraguchi, S., Suzuki, R., Yokoyama, M., Inoue, M., Aizawa, S., Toyoda, Y. and Sato, E. (1996). Meiotic abnormalities of c-*mos* knockout mouse oocytes: Activation after first meiosis or entrance into third meiotic metaphase. *Biology of Reproduction*, **55**(6), 1315–1324. doi: 10.1095/biolreprod55.6.1315

Bai, D., Sun, J., Jia, Y., Yin, J., Zhang, Y., Li, Y., Gao, R., Du, X., Li, K., Lin, J., Tu, Z., Wang, Y., Pan, J., Liang, S., Guo, Y., Ruan, J., Kou, X., Zhao, Y., Wang, H., *et al.* (2020). Genome transfer for the prevention of female infertility caused by maternal gene mutation. *Journal of Genetics and Genomics*, **47**(6), 311–319. doi: 10.1016/j.jgg.2020.06.002

Balakier, H. (1993). Tripronuclear human zygotes: The first cell cycle and subsequent development. *Human Reproduction*, **8**(11), 1892–1897. doi: 10.1093/oxfordjournals.humrep.a137955

Balakier, H., Sojecki, A., Motamedi, G. and Librach, C. (2004). Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. *Human Reproduction*, **19**(4), 982–987. doi: 10.1093/humrep/deh158

Bebbere, D., Masala, L., Albertini, D. F. and Ledda, S. (2016). The subcortical maternal complex: Multiple functions for one biological structure? *Journal of Assisted Reproduction and Genetics*, **33**(11), 1431–1438. doi: 10.1007/s10815-016-0788-z

Benkhalifa, M., Kahraman, S., Caserta, D., Domez, E. and Qumsiyeh, M. B. (2003). Morphological and cytogenetic analysis of intact oocytes and blocked zygotes. *Prenatal Diagnosis*, **23**(5), 397–404. doi: 10.1002/pd.606

Bernhardt, M. L., Padilla-Banks, E., Stein, P., Zhang, Y. and Williams, C. J. (2017). Store-operated Ca^{2+} entry is not required for fertilization-induced Ca^{2+} signaling in mouse eggs. *Cell Calcium*, **65**, 63–72. doi: 10.1016/j.ceca.2017.02.004

Capalbo, A., Poli, M., Riera-Escamilla, A., Shukla, V., Kudo Høffding, M., Krausz, C., Hoffmann, E. R. and Simon, C. (2021). Preconception genome medicine: Current state and future perspectives to improve infertility diagnosis and reproductive and health outcomes based on individual genomic data. *Human Reproduction Update*, **27**(2), 254–279. doi: 10.1093/humupd/dmaa044

Castel, S. E., Cervera, A., Mohammadi, P., Aguet, F., Reverter, F., Wolman, A., Guigo, R., Iossifov, I., Vasileva, A. and Lappalainen, T. (2018). Modified penetrance of coding variants by *cis*-regulatory variation contributes to disease risk. *Nature Genetics*, **50**(9), 1327–1334. doi: 10.1038/s41588-018-0192-y

Chemes, H. E., Puigdomenech, E. T., Carizza, C., Olmedo, S. B., Zanchetti, F. and Hermes, R. (1999). Acephalic spermatozoa and abnormal development of the head-neck attachment: A human syndrome of genetic origin. *Human Reproduction*, **14**(7), 1811–1818. doi: 10.1093/humrep/14.7.1811

Chen, B., Li, B., Li, D., Yan, Z., Mao, X., Xu, Y., Mu, J., Li, Q., Jin, L., He, L., Kuang, Y., Sang, Q. and Wang, L. (2017a). Novel mutations and structural deletions in TUBB8: Expanding mutational and phenotypic spectrum of patients with arrest in oocyte maturation, fertilization or early embryonic development. *Human Reproduction*, **32**(2), 457–464. doi: 10.1093/humrep/dew322

Chen, B., Zhang, Z., Sun, X., Kuang, Y., Mao, X., Wang, X., Yan, Z., Li, B., Xu, Y., Yu, M., Fu, J., Mu, J., Zhou, Z., Li, Q., Jin, L., He, L., Sang, Q. and Wang, L. (2017b). Biallelic mutations in PATL2 cause female infertility characterized by oocyte maturation arrest. *American Journal of Human Genetics*, **101**(4), 609–615. doi: 10.1016/j.ajhg.2017.08.018

Chen, B., Wang, W., Peng, X., Jiang, H., Zhang, S., Li, D., Li, B., Fu, J., Kuang, Y., Sun, X., Wang, X., Zhang, Z., Wu, L., Zhou, Z., Lyu, Q., Yan, Z., Mao, X., Xu, Y., Mu, J., *et al.* (2019). The comprehensive mutational and phenotypic spectrum of TUBB8 in female infertility. *European Journal of Human Genetics: EJHG*, **27**(2), 300–307. doi: 10.1038/s41431-018-0283-3

Cheung, S., Xie, P., Parrella, A., Keating, D., Rosenwaks, Z. and Palermo, G. D. (2020). Identification and treatment of men with phospholipase Cζ-defective spermatozoa. *Fertility and Sterility*, **114**(3), 535–544. doi: 10.1016/j.fertnstert.2020.04.044

Christou-Kent, M., Kherraf, Z. E., Amiri-Yekta, A., Le Blévec, E., Karaouzène, T., Conne, B., Escoffier, J., Assou, S., Guttin, A., Lambert, E., Martinez, G., Boguenet, M., Fourati Ben Mustapha, S., Cedrin Durnerin, I., Halouani, L., Marrakchi, O., Makni, M., Latrous, H., Kharouf, M., *et al.* (2018). PATL2 is a key actor of oocyte maturation whose invalidation causes infertility in women and mice. *EMBO Molecular Medicine*, **10**(5), e8515. doi: 10.15252/emmm.201708515

Combelles, C. M., Morozumi, K., Yanagimachi, R., Zhu, L., Fox, J. H. and Racowsky, C. (2010). Diagnosing cellular defects in an unexplained case of total fertilization failure. *Human Reproduction*, **25**(7), 1666–1671. doi: 10.1093/humrep/deq064

Costa-Borges, N., Spath, K., Miguel-Escalada, I., Mestres, E., Balmaseda, R., Serafín, A., Garcia-Jiménez, M., Vanrell, I., González, J., Rink, K., Wells, D. and Calderón, G. (2020). Maternal spindle transfer overcomes embryo developmental arrest caused by ooplasmic defects in mice. *eLife*, **9**, e48591. doi: 10.7554/eLife.48591

Coward, K., Ponting, C. P., Chang, H. Y., Hibbitt, O., Savolainen, P., Jones, K. T. and Parrington, J. (2005). Phospholipase Czeta, the trigger of egg activation in mammals, is present in a non-mammalian species. *Reproduction*, **130**(2), 157–163. doi: 10.1530/rep.1.00707

Dadoune, J. P., Siffroi, J. P. and Alfonsi, M. F. (2004). Transcription in haploid male germ cells. *International Review of Cytology*, **237**, 1–56. doi: 10.1016/S0074-7696(04)37001-4

Dai, J., Zhang, T., Guo, J., Zhou, Q., Gu, Y., Zhang, J., Hu, L., Zong, Y., Song, J., Zhang, S., Dai, C., Gong, F., Lu, G., Zheng, W. and Lin, G. (2021). Homozygous pathogenic variants in ACTL9 cause fertilization failure and male infertility in humans and mice. *American Journal of Human Genetics*, **108**(3), 469–481. doi: 10.1016/j.ajhg.2021.02.004

Dam, A. H., Koscinski, I., Kremer, J. A., Moutou, C., Jaeger, A. S., Oudakker, A. R., Tournaye, H., Charlet, N., Lagier-Tourenne, C., van Bokhoven, H. and Viville, S. (2007). Homozygous mutation in SPATA16 is associated with male infertility in human globozoospermia. *American Journal of Human Genetics*, **81**(4), 813–820. doi: 10.1086/521314

Darwish, E. and Magdi, Y. (2015). A preliminary report of successful cleavage after calcium ionophore activation at ICSI in cases with previous arrest at the pronuclear stage. *Reproductive Biomedicine Online*, **31**(6), 799–804. doi: 10.1016/j.rbmo.2015.08.012

Docherty, L. E., Rezwan, F. I., Poole, R. L., Turner, C. L., Kivuva, E., Maher, E. R., Smithson, S. F., Hamilton-Shield, J. P., Patalan, M., Gizewska, M., Peregud-Pogorzelski, J., Beygo, J., Buiting, K., Horsthemke, B., Soellner, L., Begemann, M., Eggermann, T., Baple, E., Mansour, S., *et al.* (2015). Mutations in NLRP5 are associated with reproductive wastage and multilocus imprinting disorders in humans. *Nature Communications*, **6**, 8086. doi: 10.1038/ncomms9086

Ducibella, T. and Fissore, R. (2008). The roles of Ca^{2+}, downstream protein kinases, and oscillatory signaling in regulating fertilization and the activation of development. *Developmental Biology*, **315**(2), 257–279. doi: 10.1016/j.ydbio.2007.12.012

Ebner, T., Moser, M., Sommergruber, M., Gaiswinkler, U., Shebl, O., Jesacher, K. and Tews, G. (2005). Occurrence and developmental consequences of vacuoles throughout preimplantation development. *Fertility and Sterility*, **83**(6), 1635–1640. doi: 10.1016/j.fertnstert.2005.02.009

Ebner, T., Oppelt, P., Wöber, M., Staples, P., Mayer, R. B., Sonnleitner, U., Bulfon-Vogl, S., Gruber, I., Haid, A. E. and Shebl, O. (2015). Treatment with Ca^{2+} ionophore improves embryo development and outcome in cases with previous developmental problems: A prospective multicenter study. *Human Reproduction*, **30**(1), 97–102. doi: 10.1093/humrep/deu285

Escoffier, J., Yassine, S., Lee, H. C., Martinez, G., Delaroche, J., Coutton, C., Karaouzène, T., Zouari, R., Metzler-Guillemain, C., Pernet-Gallay, K., Hennebicq, S., Ray, P. F., Fissore, R. and Arnoult, C. (2015). Subcellular localization of phospholipase Cζ in human sperm and its absence in DPY19L2-deficient sperm are consistent with its role in oocyte activation. *Molecular Human Reproduction*, **21**(2), 157–168. doi: 10.1093/molehr/gau098

Escoffier, J., Lee, H. C., Yassine, S., Zouari, R., Martinez, G., Karaouzène, T., Coutton, C., Kherraf, Z. E., Halouani, L., Triki, C., Nef, S., Thierry-Mieg, N., Savinov, S. N., Fissore, R., Ray, P. F. and Arnoult, C. (2016). Homozygous mutation of PLCZ1 leads to defective human oocyte activation and infertility that is not rescued by the WW-binding protein PAWP. *Human Molecular Genetics*, **25**(5), 878–891. doi: 10.1093/hmg/ddv617

Fallahi, J., Anvar, Z., Razban, V., Momtahan, M., Namavar-Jahromi, B. and Fardaei, M. (2020). Founder effect of KHDC3L, p.M1V mutation, on Iranian Patients with recurrent hydatidiform moles. *Iranian Journal of Medical Sciences*, **45**(2), 118–124. doi: 10.30476/IJMS.2019.45335, p.M1v.

Fan, Y., Zhao, H. C., Liu, J., Tan, T., Ding, T., Li, R., Zhao, Y., Yan, J., Sun, X., Yu, Y. and Qiao, J. (2015). Aberrant expression of maternal Plk1 and Dctn3 results in the developmental failure of human in-vivo- and in-vitro-matured oocytes. *Scientific Reports*, **5**, 8192. doi: 10.1038/srep08192

Feng, R., Yan, Z., Li, B., Yu, M., Sang, Q., Tian, G., Xu, Y., Chen, B., Qu, R., Sun, Z., Sun, X., Jin, L., He, L., Kuang, Y., Cowan, N. J. and Wang, L. (2016). Mutations in TUBB8 cause a multiplicity of phenotypes in human oocytes and early embryos. *Journal of Medical Genetics*, **53**(10), 662–671. doi: 10.1136/jmedgenet-2016-103891

Fernandes, R., Tsuda, C., Perumalsamy, A. L., Naranian, T., Chong, J., Acton, B. M., Tong, Z. B., Nelson, L. M. and Jurisicova, A. (2012). NLRP5 mediates mitochondrial function in mouse oocytes and embryos. *Biology of Reproduction*, **86**(5), 131–110. doi: 10.1095/biolreprod.111.093583

Ferrer-Buitrago, M., Dhaenens, L., Lu, Y., Bonte, D., Vanden Meerschaut, F., De Sutter, P., Leybaert, L. and Heindryckx, B. (2018). Human oocyte calcium analysis predicts the response to assisted oocyte activation in patients experiencing fertilization failure after ICSI. *Human Reproduction*, **33**(3), 416–425. doi: 10.1093/humrep/dex376

Ferrer-Buitrago, M., Bonte, D., Dhaenens, L., Vermorgen, S., Lu, Y., De Sutter, P. and Heindryckx, B. (2019). Assessment of the calcium releasing machinery in oocytes that failed to fertilize after conventional ICSI and assisted oocyte activation. *Reproductive Biomedicine Online*, **38**(4), 497–507. doi: 10.1016/j.rbmo.2018.12.035

Ferrer-Buitrago, M., Tilleman, L., Thys, V., Hachem, A., Boel, A., Van Nieuwerburgh, F., Deforce, D., Leybaert, L., De Sutter, P., Parrington, J. and Heindryckx, B. (2020). Comparative study of preimplantation development following distinct assisted oocyte activation protocols in a PLC-zeta knockout mouse model. *Molecular Human Reproduction*, **26**(11), 801–815. doi: 10.1093/molehr/gaaa060

Ferrer-Vaquer, A., Barragan, M., Freour, T., Vernaeve, V. and Vassena, R. (2016). PLCζ sequence, protein levels, and distribution in human sperm do not correlate with semen characteristics and fertilization rates after ICSI. *Journal of Assisted Reproduction and Genetics*, **33**(6), 747–756. doi: 10.1007/s10815-016-0718-0

Flaherty, S. P., Payne, D. and Matthews, C. D. (1998). Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection. *Human Reproduction*, 13, Suppl. **1**, 155–164. doi: 10.1093/humrep/13.suppl_1.155

Freour, T., Barragan, M., Torra-Massana, M., Ferrer-Vaquer, A. and Vassena, R. (2018). Is there an association between PAWP/WBP2NL sequence, expression, and distribution in sperm cells and fertilization failures in ICSI cycles? *Molecular Reproduction and Development*, **85**(2), 163–170. doi: 10.1002/mrd.22950

Gao, Y., Tian, S., Sha, Y., Zha, X., Cheng, H., Wang, A., Liu, C., Lv, M., Ni, X., Li, Q., Wu, H., Tan, Q., Tang, D., Song, B., Ding, D., Cong, J., Xu, Y., Zhou, P., Wei, Z., *et al.* (2021). Novel bi-allelic variants in DNAH2 cause severe asthenoteratozoospermia with multiple morphological abnormalities of the flagella. *Reproductive Biomedicine Online*, **42**(5), 963–972. doi: 10.1016/j.rbmo.2021.01.011

Gou, L. T., Lim, D. H., Ma, W., Aubol, B. E., Hao, Y., Wang, X., Zhao, J., Liang, Z., Shao, C., Zhang, X., Meng, F., Li, H., Zhang, X., Xu, R., Li, D., Rosenfeld, M. G., Mellon, P. L., Adams, J. A., Liu, M. F. and Fu, X. D. (2020). Initiation of parental genome reprogramming in fertilized oocyte by splicing kinase SRPK1-catalyzed protamine phosphorylation. *Cell*, **180**(6), 1212–1227.e14. doi: 10.1016/j.cell.2020.02.020

Guggilla, R. R. B. A., Ferrer-Buitrago, M., Bonte, D., De Sutter, P., Coucke, P. and Heindryckx, B. (2019). Heterozygous mutations in PLCZ1 are associated with fertilization failure after ICSI. O-252. *Human Reproduction*, **34**.

Hachem, A., Godwin, J., Ruas, M., Lee, H. C., Ferrer Buitrago, M., Ardestani, G., Bassett, A., Fox, S., Navarrete, F., de Sutter, P., Heindryckx, B., Fissore, R. and Parrington, J. (2017). PLCζ is the physiological trigger of the Ca^{2+} oscillations that induce embryogenesis in mammals but conception can occur in its absence. *Development*, **144**(16), 2914–2924. doi: 10.1242/dev.150227

Hamilton, L. E., Suzuki, J., Acteau, G., Shi, M., Xu, W., Meinsohn, M. C., Sutovsky, P. and Oko, R. (2018). WBP2 shares a common location in mouse spermatozoa with WBP2NL/PAWP and like its descendent is a candidate mouse oocyte-activating factor. *Biology of Reproduction*, **99**(6), 1171–1183. doi: 10.1093/biolre/ioy156

Hamilton, L. E., Suzuki, J., Aguila, L., Meinsohn, M. C., Smith, O. E., Protopapas, N., Xu, W., Sutovsky, P. and Oko, R. (2019). Sperm-borne glutathione-*S*-transferase omega 2 accelerates the nuclear decondensation of spermatozoa during fertilization in mice. *Biology of Reproduction*, **101**(2), 368–376. doi: 10.1093/biolre/ioz082

Hanna, C. B., Yao, S., Martin, M., Schönbrunn, E., Georg, G. I., Jensen, J. T. and Cuellar, R. A. D. (2019). Identification and screening of selective WEE2 inhibitors to develop non-hormonal contraceptives that specifically target meiosis. *ChemistrySelect*, **4**(45), 13363–13369. doi: 10.1002/slct.201903696

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Heindryckx, B., Van der Elst, J., De Sutter, P. and Dhont, M. (2005). Treatment option for sperm- or oocyte-related fertilization failure: Assisted oocyte activation following diagnostic heterologous ICSI. *Human Reproduction*, **20**(8), 2237–2241. doi: 10.1093/humrep/dei029

Heytens, E., Parrington, J., Coward, K., Young, C., Lambrecht, S., Yoon, S. Y., Fissore, R. A., Hamer, R., Deane, C. M., Ruas, M., Grasa, P., Soleimani, R., Cuvelier, C. A., Gerris, J., Dhont, M., Deforce, D., Leybaert, L. and De Sutter, P. (2009). Reduced amounts and abnormal forms of phospholipase C zeta (PLCzeta) in spermatozoa from infertile men. *Human Reproduction*, **24**(10), 2417–2428. doi: 10.1093/humrep/dep207

Hojnik, N. and Kovačič B. (2019). Oocyte activation failure: physiological and clinical aspects. Chapter 4. In: Bin, W. and Feng, H. L. (eds), *Embryogenesis*. IntechOpen Publishers. doi: 10.5772/intechopen.83488

Huang, L., Tong, X., Wang, F., Luo, L., Jin, R., Fu, Y., Zhou, G., Li, D., Song, G., Liu, Y. and Zhu, F. (2018). Novel mutations in PATL2 cause female infertility with oocyte germinal vesicle arrest. *Human Reproduction*, **33**(6), 1183–1190. doi: 10.1093/humrep/dey100

Huang, L., Wang, F., Kong, S., Wang, Y., Song, G., Lu, F., Ji, J., Luo, L. and Tong, X. (2021). Novel mutations in CDC20 are associated with female infertility due to oocyte maturation abnormality and early embryonic arrest. *Reproductive Sciences*, **28**(7), 1930–1938. doi: 10.1007/s43032-021-00524-3

Hwang, L. H., Lau, L. F., Smith, D. L., Mistrot, C. A., Hardwick, K. G., Hwang, E. S., Amon, A. and Murray, A. W. (1998). Budding yeast Cdc20: A target of the spindle checkpoint. *Science*, **279**(5353), 1041–1044. doi: 10.1126/science.279.5353.1041

Ilkan, A., Aktuna, S., Duman, T., Hurdag, C., Uner Ayvaz, O., Canillioglu, Y., Baltaci, V. and Unsal, E. (2014). P-446 Mutation analysis of phospholipase C zeta (PLCζ) in patients with low fertilisation rate. *Human Reproduction*, **29**(Suppl 1), i302–i303. doi: 10.1093/humrep/29.Supplement_1.1

Jha, K. N., Tripurani, S. K. and Johnson, G. R. (2017). TSSK6 is required for γH2AX formation and the histone-to-protamine transition during spermiogenesis. *Journal of Cell Science*, **130**(10), 1835–1844. doi: 10.1242/jcs.202721

Jia, M., Shi, R. and Xue, X. (2021). Novel DNAH17 mutations associated with fertilization failures after ICSI. *Gynecological Endocrinology*, **37**(8), 769–771. doi: 10.1080/09513590.2021.1937979

Kashir, J., Konstantinidis, M., Jones, C., Lemmon, B., Lee, H. C., Hamer, R., Heindryckx, B., Deane, C. M., De Sutter, P., Fissore, R. A., Parrington, J., Wells, D. and Coward, K. (2012). A maternally inherited autosomal point mutation in human phospholipase C zeta (PLCζ) leads to male infertility. *Human Reproduction*, **27**(1), 222–231. doi: 10.1093/humrep/der384

Kim, B., Zhang, X., Kan, R., Cohen, R., Mukai, C., Travis, A. J. and Coonrod, S. A. (2014). The role of MATER in endoplasmic reticulum distribution and calcium homeostasis in mouse oocytes. *Developmental Biology*, **386**(2), 331–339. doi: 10.1016/j.ydbio.2013.12.025

Kline, D., Mehlmann, L., Fox, C. and Terasaki, M. (1999). The cortical endoplasmic reticulum (ER) of the mouse egg: Localization of ER clusters in relation to the generation of repetitive calcium waves. *Developmental Biology*, **215**(2), 431–442. doi: 10.1006/dbio.1999.9445

Kouchi, Z., Shikano, T., Nakamura, Y., Shirakawa, H., Fukami, K. and Miyazaki, S. (2005). The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase Czeta. *Journal of Biological Chemistry*, **280**(22), 21015–21021. doi: 10.1074/jbc.M412123200

Kraft, C., Vodermaier, H. C., Maurer-Stroh, S., Eisenhaber, F. and Peters, J. M. (2005). The WD40 propeller domain of Cdh1 functions as a destruction box receptor for APC/C substrates. *Molecular Cell*, **18**(5), 543–553. doi: 10.1016/j.molcel.2005.04.023

Lazaros, L. A., Vartholomatos, G. A., Hatzi, E. G., Kaponis, A. I., Makrydimas, G. V., Kalantaridou, S. N., Sofikitis, N. V., Stefos, T. I., Zikopoulos, K. A. and Georgiou, I. A. (2011). Assessment of sperm chromatin condensation and ploidy status using flow cytometry correlates to fertilization, embryo quality and pregnancy following *in vitro* fertilization. *Journal of Assisted Reproduction and Genetics*, **28**(10), 885–891. doi: 10.1007/s10815-011-9611-z

Li, L., Baibakov, B. and Dean, J. (2008). A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Developmental Cell*, **15**(3), 416–425. doi: 10.1016/j.devcel.2008.07.010

Li, M., Jia, M., Zhao, X., Shi, R. and Xue, X. (2021). A new NLRP5 mutation causes female infertility and total fertilization failure. *Gynecological Endocrinology*, **37**(3), 283–284. doi: 10.1080/09513590.2020.1832069

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., Clarke, M., Devereaux, P. J., Kleijnen, J. and Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLOS Medicine*, **6**(7), e1000100. doi: 10.1371/journal.pmed.1000100

Lin, J., Xu, H., Chen, B., Wang, W., Wang, L., Sun, X. and Sang, Q. (2020). Expanding the genetic and phenotypic spectrum of female infertility caused by TLE6 mutations. *Journal of Assisted Reproduction and Genetics*, **37**(2), 437–442. doi: 10.1007/s10815-019-01653-0

Liu, C., Miyata, H., Gao, Y., Sha, Y., Tang, S., Xu, Z., Whitfield, M., Patrat, C., Wu, H., Dulioust, E., Tian, S., Shimada, K., Cong, J., Noda, T., Li, H., Morohoshi, A., Cazin, C., Kherraf, Z. E., Arnoult, C., *et al.* (2020). Bi-allelic DNAH8 variants lead to multiple morphological abnormalities of the sperm flagella and primary male infertility. *American Journal of Human Genetics*, **107**(2), 330–341. doi: 10.1016/j.ajhg.2020.06.004

Liu, M., Sun, Y., Li, Y., Sun, J., Yang, Y. and Shen, Y. (2021). Novel mutations in FSIP2 lead to multiple morphological abnormalities of the sperm flagella and poor ICSI prognosis. *Gene*, **781**, 145536. doi: 10.1016/j.gene.2021.145536

Maddirevula, S., Coskun, S., Awartani, K., Alsaif, H., Abdulwahab, F. M. and Alkuraya, F. S. (2017). The human knockout phenotype of PADI6 is female sterility caused by cleavage failure of their fertilized eggs. *Clinical Genetics*, **91**(2), 344–345. doi: 10.1111/cge.12866

Maddirevula, S., Awartani, K., Coskun, S., AlNaim, L. F., Ibrahim, N., Abdulwahab, F., Hashem, M., Alhassan, S. and Alkuraya, F. S. (2020). A genomics approach to females with infertility and recurrent pregnancy loss. *Human Genetics*, **139**(5), 605–613. doi: 10.1007/s00439-020-02143-5

Moos, J., Visconti, P. E., Moore, G. D., Schultz, R. M. and Kopf, G. S. (1995). Potential role of mitogen-activated protein kinase in pronuclear envelope assembly and disassembly following fertilization of mouse eggs. *Biology of Reproduction*, **53**(3), 692–699. doi: 10.1095/biolreprod53.3.692

Mu, J., Zhang, Z., Wu, L., Fu, J., Chen, B., Yan, Z., Li, B., Zhou, Z., Wang, W., Zhao, L., Dong, J., Kuang, Y., Sun, X., He, L., Wang, L. and Sang, Q. (2020). The identification of novel mutations in PLCZ1 responsible for human fertilization failure and a therapeutic intervention by artificial oocyte activation. *Molecular Human Reproduction*, **26**(2), 80–87. doi: 10.1093/molehr/gaaa003

Nakamura, Y., Tanaka, K. J., Miyauchi, M., Huang, L., Tsujimoto, M. and Matsumoto, K. (2010). Translational repression by the oocyte-specific protein P100 in Xenopus. *Developmental Biology*, **344**(1), 272–283. doi: 10.1016/j.ydbio.2010.05.006

Nikiforaki, D., Vanden Meerschaut, F., De Gheselle, S., Qian, C., Van den Abbeel, E., De Vos, W. H., Deroo, T., De Sutter, P. and Heindryckx, B. (2014). Sperm involved in recurrent partial hydatidiform moles cannot induce the normal pattern of calcium oscillations. *Fertility and Sterility*, **102**(2), 581–588.e1 e581. doi: 10.1016/j.fertnstert.2014.05.004

Nomikos, M., Elgmati, K., Theodoridou, M., Calver, B. L., Nounesis, G., Swann, K. and Lai, F. A. (2011). Phospholipase Cζ binding to PtdIns(4,5)P2 requires the XY-linker region. *Journal of Cell Science*, **124**(15), 2582–2590. doi: 10.1242/jcs.083485

Nomikos, M., Yu, Y., Elgmati, K., Theodoridou, M., Campbell, K., Vassilakopoulou, V., Zikos, C., Livaniou, E., Amso, N., Nounesis, G., Swann, K. and Lai, F. A. (2013). Phospholipase Cζ rescues failed oocyte activation in a prototype of male factor infertility. *Fertility and Sterility*, **99**(1), 76–85. doi: 10.1016/j.fertnstert.2012.08.035

Nomikos, M., Sanders, J. R., Theodoridou, M., Kashir, J., Matthews, E., Nounesis, G., Lai, F. A. and Swann, K. (2014). Sperm-specific post-acrosomal WW-domain binding protein (PAWP) does not cause Ca^{2+} release in mouse oocytes. *Molecular Human Reproduction*, **20**(10), 938–947. doi: 10.1093/molehr/gau056

Nomikos, M., Stamatiadis, P., Sanders, J. R., Beck, K., Calver, B. L., Buntwal, L., Lofty, M., Sideratou, Z., Swann, K. and Lai, F. A. (2017). Male infertility-linked point mutation reveals a vital binding role for the C2 domain of

sperm PLCζ. *Biochemical Journal*, **474**(6), 1003–1016. doi: 10.1042/BCJ20161057

Nozawa, K., Satouh, Y., Fujimoto, T., Oji, A. and Ikawa, M. (2018). Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice. *Scientific Reports*, **8**(1), 1315. doi: 10.1038/s41598-018-19497-6

Oh, J. S., Han, S. J. and Conti, M. (2010). Wee1B, Myt1, and Cdc25 function in distinct compartments of the mouse oocyte to control meiotic resumption. *Journal of Cell Biology*, **188**(2), 199–207. doi: 10.1083/jcb.200907161

Oh, J. S., Susor, A. and Conti, M. (2011). Protein tyrosine kinase Wee1B is essential for metaphase II exit in mouse oocytes. *Science*, **332**(6028), 462–465. doi: 10.1126/science.1199211

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A. C. (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340**(8810), 17–18. doi: 10.1016/0140-6736(92)92425-f

Palermo, G. D., O'Neill, C. L., Chow, S., Cheung, S., Parrella, A., Pereira, N. and Rosenwaks, Z. (2017). Intracytoplasmic sperm injection: State of the art in humans. *Reproduction*, **154**(6), F93–F110. doi: 10.1530/REP-17-0374

Proell, M., Riedl, S. J., Fritz, J. H., Rojas, A. M. and Schwarzenbacher, R. (2008). The Nod-like receptor (NLR) family: A tale of similarities and differences. *PLOS ONE*, **3**(4), e2119. doi: 10.1371/journal.pone.0002119

Pujol, A., García, D., Obradors, A., Rodríguez, A. and Vassena, R. (2018). Is there a relation between the time to ICSI and the reproductive outcomes? *Human Reproduction*, **33**(5), 797–806. doi: 10.1093/humrep/dey067

Qian, J., Nguyen, N. M. P., Rezaei, M., Huang, B., Tao, Y., Zhang, X., Cheng, Q., Yang, H., Asangla, A., Majewski, J. and Slim, R. (2018). Biallelic PADI6 variants linking infertility, miscarriages, and hydatidiform moles. *European Journal of Human Genetics: EJHG*, **26**(7), 1007–1013. doi: 10.1038/s41431-018-0141-3

Rawe, V. Y., Díaz, E. S., Abdelmassih, R., Wójcik, C., Morales, P., Sutovsky, P. and Chemes, H. E. (2008). The role of sperm proteasomes during sperm aster formation and early zygote development: Implications for fertilization failure in humans. *Human Reproduction*, **23**(3), 573–580. doi: 10.1093/humrep/dem385

Rawe, V. Y., Olmedo, S. B., Nodar, F. N., Doncel, G. D., Acosta, A. A. and Vitullo, A. D. (2000). Cytoskeletal organization defects and abortive activation in human oocytes after IVF and ICSI failure. *Molecular Human Reproduction*, **6**(6), 510–516. doi: 10.1093/molehr/6.6.510

Rawe, V. Y., Terada, Y., Nakamura, S., Chillik, C. F., Olmedo, S. B. and Chemes, H. E. (2002). A pathology of the sperm centriole responsible for defective sperm aster formation, syngamy and cleavage. *Human Reproduction*, **17**(9), 2344–2349. doi: 10.1093/humrep/17.9.2344

Revenkova, E., Eijpe, M., Heyting, C., Hodges, C. A., Hunt, P. A., Liebe, B., Scherthan, H. and Jessberger, R. (2004). Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. *Nature Cell Biology*, **6**(6), 555–562. doi: 10.1038/ncb1135

Rogers, N. T., Hobson, E., Pickering, S., Lai, F. A., Braude, P. and Swann, K. (2004). Phospholipase Czeta causes Ca^{2+} oscillations and parthenogenetic activation of human oocytes. *Reproduction*, **128**(6), 697–702. doi: 10.1530/rep.1.00484

Roig, I., Dowdle, J. A., Toth, A., de Rooij, D. G., Jasin, M. and Keeney, S. (2010). Mouse TRIP13/PCH2 is required for recombination and normal higher-order chromosome structure during meiosis. *PLOS Genetics*, **6**(8). doi: 10.1371/journal.pgen.1001062

Rybouchkin, A., Dozortsev, D., Pelinck, M. J., De Sutter, P. and Dhont, M. (1996). Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes. *Human Reproduction*, **11**(10), 2170–2175. doi: 10.1093/oxfordjournals.humrep.a019071

Sadowy, S., Tomkin, G., Munné, S., Ferrara-Congedo, T. and Cohen, J. (1998). Impaired development of zygotes with uneven pronuclear size. *Zygote*, **6**(2), 137–141. doi: 10.1017/s0967199498000057

Sanders, J. R., Ashley, B., Moon, A., Woolley, T. E. and Swann, K. (2018). PLCζ induced Ca^{2+} oscillations in mouse eggs involve a positive feedback cycle of Ca^{2+} induced InsP3 formation from cytoplasmic PIP2. *Frontiers in Cell and Developmental Biology*, **6**, 36. doi: 10.3389/fcell.2018.00036

Sang, Q., Li, B., Kuang, Y., Wang, X., Zhang, Z., Chen, B., Wu, L., Lyu, Q., Fu, Y., Yan, Z., Mao, X., Xu, Y., Mu, J., Li, Q., Jin, L., He, L. and Wang, L. (2018). Homozygous mutations in WEE2 cause fertilization failure and female infertility. *American Journal of Human Genetics*, **102**(4), 649–657. doi: 10.1016/j.ajhg.2018.02.015

Satouh, Y., Nozawa, K. and Ikawa, M. (2015). Sperm postacrosomal WW domain-binding protein is not required for mouse egg activation. *Biology of Reproduction*, **93**(4), 94. doi: 10.1095/biolreprod.115.131441

Saunders, C. M., Larman, M. G., Parrington, J., Cox, L. J., Royse, J., Blayney, L. M., Swann, K. and Lai, F. A. (2002). PLC zeta: A sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development*, **129**(15), 3533–3544. doi: 10.1242/dev.129.15.3533

Sousa, M. and Tesarik, J. (1994). Ultrastructural analysis of fertilization failure after intracytoplasmic sperm injection. *Human Reproduction*, **9**(12), 2374–2380. doi: 10.1093/oxfordjournals.humrep.a138455

Tachibana, M., Terada, Y., Ogonuki, N., Ugajin, T., Ogura, A., Murakami, T., Yaegashi, N. and Okamura, K. (2009). Functional assessment of centrosomes of spermatozoa and spermatids microinjected into rabbit oocytes. *Molecular Reproduction and Development*, **76**(3), 270–277. doi: 10.1002/mrd.20951

Tashiro, F., Kanai-Azuma, M., Miyazaki, S., Kato, M., Tanaka, T., Toyoda, S., Yamato, E., Kawakami, H., Miyazaki, T. and Miyazaki, J. (2010). Maternal-effect gene Ces5/Ooep/Moep19/Floped is essential for oocyte cytoplasmic lattice formation and embryonic development at the maternal-zygotic stage transition. *Genes to Cells: Devoted to Molecular and Cellular Mechanisms*, **15**(8), 813–828. doi: 10.1111/j.1365-2443.2010.01420.x

Tian, Y., Wang, G., Wang, J., Mu, X., Chen, H., Song, X. and Bai, X. (2020). Novel compound heterozygous mutation in WEE2 is associated with fertilization failure: case report of an infertile woman and literature review. *BMC Womens Health*, **20**(1), 246. doi: 10.1186/s12905-020-01111-5

Torra-Massana, M., Cornet-Bartolomé, D., Barragán, M., Durban, M., Ferrer-Vaquer, A., Zambelli, F., Rodriguez, A., Oliva, R. and Vassena, R. (2019). Novel phospholipase C zeta 1 mutations associated with fertilization failures after ICSI. *Human Reproduction*, **34**(8), 1494–1504. doi: 10.1093/humrep/dez094

Tu, C., Cong, J., Zhang, Q., He, X., Zheng, R., Yang, X., Gao, Y., Wu, H., Lv, M., Gu, Y., Lu, S., Liu, C., Tian, S., Meng, L., Wang, W., Tan, C., Nie, H., Li, D., Zhang, H., *et al.* (2021). Bi-allelic mutations of DNAH10 cause primary male infertility with asthenoteratozoospermia in humans and mice. *American Journal of Human Genetics*, **108**(8), 1466–1477. doi: 10.1016/j.ajhg.2021.06.010

Vanden Meerschaut, F., Nikiforaki, D., De Gheselle, S., Dullaerts, V., Van den Abbeel, E., Gerris, J., Heindryckx, B. and De Sutter, P. (2012). Assisted oocyte activation is not beneficial for all patients with a suspected oocyte-related activation deficiency. *Human Reproduction*, **27**(7), 1977–1984. doi: 10.1093/humrep/des097

Vanden Meerschaut, F., Leybaert, L., Nikiforaki, D., Qian, C., Heindryckx, B. and De Sutter, P. (2013). Diagnostic and prognostic value of calcium oscillatory pattern analysis for patients with ICSI fertilization failure. *Human Reproduction*, **28**(1), 87–98. doi: 10.1093/humrep/des368

Vassena, R., Boué, S., González-Roca, E., Aran, B., Auer, H., Veiga, A. and Izpisua Belmonte, J. C. (2011). Waves of early transcriptional activation and pluripotency program initiation during human preimplantation development. *Development*, **138**(17), 3699–3709. doi: 10.1242/dev.064741

Wambergue, C., Zouari, R., Fourati Ben Mustapha, S., Martinez, G., Devillard, F., Hennebicq, S., Satre, V., Brouillet, S., Halouani, L., Marrakchi, O., Makni, M., Latrous, H., Kharouf, M., Amblard, F., Arnoult, C., Ray, P. F. and Coutton, C. (2016). Patients with multiple morphological abnormalities of the sperm flagella due to DNAH1 mutations have a good prognosis following intracytoplasmic sperm injection. *Human Reproduction*, **31**(6), 1164–1172. doi: 10.1093/humrep/dew083

Wang, W., Tu, C., Nie, H., Meng, L., Li, Y., Yuan, S., Zhang, Q., Du, J., Wang, J., Gong, F., Fan, L., Lu, G. X., Lin, G. and Tan, Y. Q. (2019). Biallelic mutations in CFAP65 lead to severe asthenoteratospermia due to acrosome hypoplasia and flagellum malformations. *Journal of Medical Genetics*, **56**(11), 750–757. doi: 10.1136/jmedgenet-2019-106031

Wang, F., Zhang, J., Kong, S., Li, C., Zhang, Z., He, X., Wu, H., Tang, D., Zha, X., Tan, Q., Duan, Z., Cao, Y. and Zhu, F. (2020). A homozygous nonsense mutation of PLCZ1 cause male infertility with oocyte activation deficiency. *Journal of Assisted Reproduction and Genetics*, **37**(4), 821–828. doi: 10.1007/s10815-020-01719-4

https://doi.org/10.1017/S096719942300014X Published online by Cambridge University Press

Wang, J., Zhang, J., Sun, X., Lin, Y., Cai, L., Cui, Y., Liu, J., Liu, M. and Yang, X. (2021). Novel bi-allelic variants in ACTL7A are associated with male infertility and total fertilization failure. *Human Reproduction*, **36**(12), 3161–3169. doi: 10.1093/humrep/deab228

Wei, X., Sha, Y., Wei, Z., Zhu, X., He, F., Zhang, X., Liu, W., Wang, Y. and Lu, Z. (2021). Bi-allelic mutations in DNAH7 cause asthenozoospermia by impairing the integrality of axoneme structure. *Acta Biochimica et Biophysica Sinica*, **53**(10), 1300–1309. doi: 10.1093/abbs/gmab113

Whitfield, M., Thomas, L., Bequignon, E., Schmitt, A., Stouvenel, L., Montantin, G., Tissier, S., Duquesnoy, P., Copin, B., Chantot, S., Dastot, F., Faucon, C., Barbotin, A. L., Loyens, A., Siffroi, J. P., Papon, J. F., Escudier, E., Amselem, S., Mitchell, V., *et al.* (2019). Mutations in DNAH17, encoding a sperm-specific axonemal outer dynein arm heavy chain, cause isolated male infertility due to asthenozoospermia. *American Journal of Human Genetics*, **105**(1), 198–212. doi: 10.1016/j.ajhg.2019.04.015

Wu, X., Viveiros, M. M., Eppig, J. J., Bai, Y., Fitzpatrick, S. L. and Matzuk, M. M. (2003). Zygote arrest 1 (*Zar1*) is a novel maternal-effect gene critical for the oocyte-to-embryo transition. *Nature Genetics*, **33**(2), 187–191. doi: 10.1038/ng1079

Wu, A. T., Sutovsky, P., Manandhar, G., Xu, W., Katayama, M., Day, B. N., Park, K. W., Yi, Y. J., Xi, Y. W., Prather, R. S. and Oko, R. (2007). PAWP, a sperm-specific WW domain-binding protein, promotes meiotic resumption and pronuclear development during fertilization. *Journal of Biological Chemistry*, **282**(16), 12164–12175. doi: 10.1074/jbc.M609132200

Wu, L., Chen, H., Li, D., Song, D., Chen, B., Yan, Z., Lyu, Q., Wang, L., Kuang, Y., Li, B. and Sang, Q. (2019). Novel mutations in PATL2: Expanding the mutational spectrum and corresponding phenotypic variability associated with female infertility. *Journal of Human Genetics*, **64**(5), 379–385. doi: 10.1038/s10038-019-0568-6

Xu, Y., Shi, Y., Fu, J., Yu, M., Feng, R., Sang, Q., Liang, B., Chen, B., Qu, R., Li, B., Yan, Z., Mao, X., Kuang, Y., Jin, L., He, L., Sun, X. and Wang, L. (2016). Mutations in PADI6 cause female infertility characterized by early embryonic arrest. *American Journal of Human Genetics*, **99**(3), 744–752. doi: 10.1016/j.ajhg.2016.06.024

Xu, Y., Qian, Y., Liu, Y., Wang, Q., Wang, R., Zhou, Y., Zhang, C., Pang, Z., Ye, H., Xue, S. and Sun, L. (2020). A novel homozygous variant in NLRP5 is associate with human early embryonic arrest in a consanguineous Chinese family. *Clinical Genetics*, **98**(1), 69–73. doi: 10.1111/cge.13744

Xu, Y., Zhu, X., Wang, M., Cai, L., Ge, Q., Fu, Y. and Jin, L. (2021). The homozygous p. Tyr228Cys variant in CDC20 causes oocyte maturation arrest: An additional evidence supporting the causality between CDC20 mutation and female infertility. *Journal of Assisted Reproduction and Genetics*, **38**(8), 2219–2222. doi: 10.1007/s10815-021-02269-z

Yamaguchi, T., Ito, M., Kuroda, K., Takeda, S. and Tanaka, A. (2017). The establishment of appropriate methods for egg-activation by human PLCZ1 RNA injection into human oocyte. *Cell Calcium*, **65**, 22–30. doi: 10.1016/j.ceca.2017.03.002

Yan, Z., Fan, Y., Wang, F., Yan, Z., Li, M., Ouyang, J., Wu, L., Yin, M., Zhao, J., Kuang, Y., Li, B. and Lyu, Q. (2020). Novel mutations in PLCZ1 cause male infertility due to fertilization failure or poor fertilization. *Human Reproduction*, **35**(2), 472–481. doi: 10.1093/humrep/dez282

Yang, X., Shu, L., Cai, L., Sun, X., Cui, Y. and Liu, J. (2019). Homozygous missense mutation Arg207Cys in the WEE2 gene causes female infertility and fertilization failure. *Journal of Assisted Reproduction and Genetics*, **36**(5), 965–971. doi: 10.1007/s10815-019

Yang, P., Yin, C., Li, M., Ma, S., Cao, Y., Zhang, C., Chen, T. and Zhao, H. (2021). Mutation analysis of tubulin beta 8 class VIII in infertile females with oocyte or embryonic defects. *Clinical Genetics*, **99**(1), 208–214. doi: 10.1111/cge.13855-01418-9

Yazawa, H., Yanagida, K., Katayose, H., Hayashi, S. and Sato, A. (2000). Comparison of oocyte activation and Ca^{2+} oscillation-inducing abilities of round/elongated spermatids of mouse, hamster, rat, rabbit and human assessed by mouse oocyte activation assay. *Human Reproduction*, **15**(12), 2582–2590. doi: 10.1093/humrep/15.12.2582

Yeste, M., Jones, C., Amdani, S. N., Patel, S. and Coward, K. (2016). Oocyte activation deficiency: A role for an oocyte contribution? *Human Reproduction Update*, **22**(1), 23–47. doi: 10.1093/humupd/dmv040

Yoon, S. Y., Jellerette, T., Salicioni, A. M., Lee, H. C., Yoo, M. S., Coward, K., Parrington, J., Grow, D., Cibelli, J. B., Visconti, P. E., Mager, J. and Fissore, R. A. (2008). Human sperm devoid of PLC, zeta 1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. *Journal of Clinical Investigation*, **118**(11), 3671–3681. doi: 10.1172/JCI36942

Yoon, S. Y., Eum, J. H., Lee, J. E., Lee, H. C., Kim, Y. S., Han, J. E., Won, H. J., Park, S. H., Shim, S. H., Lee, W. S., Fissore, R. A., Lee, D. R. and Yoon, T. K. (2012). Recombinant human phospholipase C zeta 1 induces intracellular calcium oscillations and oocyte activation in mouse and human oocytes. *Human Reproduction*, **27**(6), 1768–1780. doi: 10.1093/humrep/des092

Yu, C., Ji, S. Y., Sha, Q. Q., Dang, Y., Zhou, J. J., Zhang, Y. L., Liu, Y., Wang, Z. W., Hu, B., Sun, Q. Y., Sun, S. C., Tang, F. and Fan, H. Y. (2016). BTG4 is a meiotic cell cycle-coupled maternal-zygotic-transition licensing factor in oocytes. *Nature Structural and Molecular Biology*, **23**(5), 387–394. doi: 10.1038/nsmb.3204

Yuan, P., Zheng, L., Liang, H., Li, Y., Zhao, H., Li, R., Lai, L., Zhang, Q. and Wang, W. (2018). A novel mutation in the TUBB8 gene is associated with complete cleavage failure in fertilized eggs. *Journal of Assisted Reproduction and Genetics*, **35**(7), 1349–1356. doi: 10.1007/s10815-018-1188-3

Yuan, P., Yang, C., Ren, Y., Yan, J., Nie, Y., Yan, L. and Qiao, J. (2020). A novel homozygous mutation of phospholipase C zeta leading to defective human oocyte activation and fertilization failure. *Human Reproduction*, **35**(4), 977–985. doi: 10.1093/humrep/dez293

Yurttas, P., Vitale, A. M., Fitzhenry, R. J., Cohen-Gould, L., Wu, W., Gossen, J. A. and Coonrod, S. A. (2008). Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. *Development*, **135**(15), 2627–2636. doi: 10.1242/dev.016329

Zamora, R. B., Sánchez, R. V., Pérez, J. G., Díaz, R. R., Quintana, D. B. and Bethencourt, J. C. (2011). Human zygote morphological indicators of higher rate of arrest at the first cleavage stage. *Zygote*, **19**(4), 339–344. doi: 10.1017/S0967199410000407

Zeng, Y., Shi, J., Xu, S., Shi, R., Wu, T., Li, H., Xue, X., Zhu, Y., Chen, B., Sang, Q. and Wang, L. (2022). Bi-allelic mutations in MOS cause female infertility characterized by preimplantation embryonic arrest. *Human Reproduction*, **37**(3), 612–620. doi: 10.1093/humrep/deab281

Zhang, J., Liu, H., Luo, S., Lu, Z., Chávez-Badiola, A., Liu, Z., Yang, M., Merhi, Z., Silber, S. J., Munné, S., Konstantinidis, M., Wells, D., Tang, J. J. and Huang, T. (2017). Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. *Reproductive Biomedicine Online*, **34**(4), 361–368. doi: 10.1016/j.rbmo.2017.01.013

Zhang, Z., Mu, J., Zhao, J., Zhou, Z., Chen, B., Wu, L., Yan, Z., Wang, W., Zhao, L., Dong, J., Sun, X., Kuang, Y., Li, B., Wang, L. and Sang, Q. (2019). Novel mutations in WEE2: Expanding the spectrum of mutations responsible for human fertilization failure. *Clinical Genetics*, **95**(4), 520–524. doi: 10.1111/cge.13505

Zhang, Z., Li, B., Fu, J., Li, R., Diao, F., Li, C., Chen, B., Du, J., Zhou, Z., Mu, J., Yan, Z., Wu, L., Liu, S., Wang, W., Zhao, L., Dong, J., He, L., Liang, X., Kuang, Y., *et al.* (2020). Bi-allelic missense pathogenic variants in TRIP13 cause female infertility characterized by oocyte maturation arrest. *American Journal of Human Genetics*, **107**(1), 15–23. doi: 10.1016/j.ajhg.2020.05.001

Zhang, Y. L., Zheng, W., Ren, P., Hu, H., Tong, X., Zhang, S. P., Li, X., Wang, H., Jiang, J. C., Jin, J., Yang, W., Cao, L., He, Y., Ma, Y., Zhang, Y., Gu, Y., Hu, L., Luo, K., Gong, F., *et al.* (2021). Biallelic mutations in MOS cause female infertility characterized by human early embryonic arrest and fragmentation. *EMBO Molecular Medicine*, **13**(12), e14887. doi: 10.15252/emmm.202114887

Zhao, S., Chen, T., Yu, M., Bian, Y., Cao, Y., Ning, Y., Su, S., Zhang, J. and Zhao, S. (2019). Novel WEE2 gene variants identified in patients with fertilization failure and female infertility. *Fertility and Sterility*, **111**(3), 519–526. doi: 10.1016/j.fertnstert.2018.11.018

Zhao, L., Guan, Y., Meng, Q., Wang, W., Wu, L., Chen, B., Hu, J., Zhu, J., Zhang, Z., Mu, J., Chen, Y., Sun, Y., Wu, T., Wang, W., Zhou, Z., Dong, J., Zeng, Y., Liu, R., Li, Q., *et al.* (2021). Identification of novel mutations in CDC20: Expanding the mutational spectrum for female infertility. *Frontiers in Cell and Developmental Biology*, **9**, 647130. doi: 10.3389/fcell.2021.647130

Zheng, W., Chen, L., Dai, J., Dai, C., Guo, J., Lu, C., Gong, F., Lu, G. and Lin, G. (2020a). New biallelic mutations in PADI6 cause recurrent preimplantation embryonic arrest characterized by direct cleavage. *Journal of Assisted Reproduction and Genetics*, **37**(1), 205–212. doi: 10.1007/s10815-019-01606-7

Zheng, W., Zhou, Z., Sha, Q., Niu, X., Sun, X., Shi, J., Zhao, L., Zhang, S., Dai, J., Cai, S., Meng, F., Hu, L., Gong, F., Li, X., Fu, J., Shi, R., Lu, G., Chen, B., Fan, H., *et al.* (2020b). Homozygous mutations in BTG4 cause zygotic cleavage failure and female infertility. *American Journal of Human Genetics*, **107**(1), 24–33. doi: 10.1016/j.ajhg.2020.05.010

Zheng, R., Sun, Y., Jiang, C., Chen, D., Yang, Y. and Shen, Y. (2021a). A novel mutation in DNAH17 is present in a patient with multiple morphological abnormalities of the flagella. *Reproductive Biomedicine Online*, **43**(3), 532–541. doi: 10.1016/j.rbmo.2021.05.009

Zheng, W., Hu, H., Dai, J., Zhang, S., Gu, Y., Dai, C., Guo, J., Xu, X., Li, Y., Zhang, S., Hu, L., Gong, F., Lu, G. and Lin, G. (2021b). Expanding the genetic and phenotypic spectrum of the subcortical maternal complex genes in recurrent preimplantation embryonic arrest. *Clinical Genetics*, **99**(2), 286–291. doi: 10.1111/cge.13858

Zhou, X. Wang, J., Liu, Z., Zhang, D., Jin, L. and Zhang, X. (2019). Novel compound heterozygous mutations in WEE2 causes female infertility and fertilization failure. *Journal of Assisted Reproduction and Genetics*, **36**(9), 1957–1962. doi: 10.1007/s10815-019-01553-3