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REVIEW

Biochemical alterations in the oocyte in support of early embryonic development

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Abstract Notwithstanding the enormous reproductive potential encapsulated within a mature mammalian oocyte, these cells present only a limited window for fertilization before defaulting to an apoptotic cascade known as post-ovulatory oocyte aging. The only cell with the capacity to rescue this potential is the fertilizing spermatozoon. Indeed, the union of these cells sets in train a remarkable series of events that endows the oocyte with the capacity to divide and differentiate into the trillions of cells that comprise a new individual. Traditional paradigms hold that, beyond the initial stimulation of fluctuating calcium (Ca^{2+}) required for oocyte activation, the fertilizing spermatozoon plays limited additional roles in the early embryo. While this model has now been drawn into question in view of the recent discovery that spermatozoa deliver developmentally important classes of small non-coding RNAs and other epigenetic modulators to oocytes during fertilization, it is nevertheless apparent that the primary responsibility for oocyte activation rests with a modest store of maternally derived proteins and mRNA accumulated during oogenesis. It is, therefore, not surprising that widespread post-translational modifications, in particular phosphorylation, hold a central role in endowing these proteins with sufficient functional diversity to initiate embryonic development. Indeed, proteins targeted for such modifications have been linked to oocyte activation, recruitment of maternal mRNAs, DNA repair and resumption of the cell cycle. This review, therefore, seeks to explore the intimate relationship between Ca^{2+} release and the suite of molecular modifications that sweep through the oocyte to ensure the successful union of the parental germlines and ensure embryogenic fidelity.

Keywords Zygote · Oocyte activation · Phosphorylation · Protein kinase · DNA repair · DNA protection

Introduction

A defining feature of the mature, ovulated, metaphase II (MII) oocyte is the narrow window of opportunity that it presents to undergo successful fertilization and initiate embryogenesis [1]. Indeed, without the union of an oocyte and a functionally mature spermatozoon, the oocyte will rapidly undergo apoptosis and degradation via a process known as post-ovulatory oocyte aging [2]. Immediately following fertilization, however, the fertilizing sperm cell initiates a series of irreversible biochemical and physiological modifications to the oocyte's cortex and cytoplasm, thus rescuing the cell from its otherwise predestined apoptotic fate and immortalizing its genetic contribution within the conceptus [3, 4]. Accordingly, the molecular basis of the sequential interactions between the fertilizing spermatozoon, the oocyte, and the subsequent events that they set in train has been the subject of considerable attention spanning many decades. Such intense research effort has provided compelling evidence that gamete fusion is followed by a rapid release of intracellular calcium from internal stores [5]. This initial elevation generally occurs within 1–3 min post-fusion [6] and, in turn, stimulates oscillating Ca^{2+} transients that can persist for a further

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3–4 h [7, 8]. It is during this period of fluctuating Ca^{2+} transients that oocyte activation is initiated (reviewed in [9]).

Coinciding with pronuclear formation and syngamy, these Ca^{2+} oscillations briefly pause, only to transiently resume during the first phase of mitosis [7]. Recent work has provided evidence that the pause in calcium ion fluxes may also occur synchronously with a critical round of DNA repair and a concomitant upregulation of protective machinery [10, 11]. Such mechanisms have been postulated to ensure the zygote is in optimal condition to undergo embryogenesis [12] and appear to be causally linked to the post-translational modification, and hence activation, of an impressive suite of reparative and protective enzymes [10]. Notwithstanding these exciting data, the identity of a majority of the targets, the complex signaling pathways that underpin their activation, and their diverse roles in preparing the oocyte for embryogenesis remain to be fully investigated and, in some instances, are the subject of considerable controversy [13, 14]. In this review, we seek to integrate the leading theories and emerging data to provide a comprehensive appraisal of the role of fertilization and oocyte activation in extending the viability of the oocyte with a focus on highlighting the prominent role of post translation modifications (PTMs), in particular phosphorylation, in these events.

Post-ovulatory oocyte aging

Immediately following ovulation into the female reproductive tract, the mature oocyte remains viable for a relatively short period of time ($\sim$16 h) before defaulting to a terminal pathway of post-ovulatory aging [4, 15]. Oocytes progressing through this degradative process bear the well-characterized hallmarks of apoptosis and recent literature has implicated mitochondrial dysfunction and a consequential generation of reactive oxygen species (ROS) as a key mediator of this process [2, 4, 15, 16]. Produced as an intermediary product of normal cellular metabolism, ROS are well known to play fundamental roles in physiological signaling [17–20]. However, imbalances created by elevated levels of ROS that overwhelm the inherent antioxidant defenses within a cell can lead to the oxidation and alkylation of cellular components, such as DNA, lipids, and proteins with dire consequences for cell viability [21, 22]. Post-ovulatory oocyte aging appears to be orchestrated, at least in part, by these oxidative processes that progress through a cascade of events encompassing elevated ROS and the peroxidation of lipids comprising the cellular membranes (not excluding the mitochondrial membrane) [2, 23]. The ensuing production of electrophilic lipid aldehydes leads to widespread adduction of vulnerable proteins and DNA causing extensive cellular damage [21]. Even prior to the final induction of apoptosis, these profound insults prevent, or severely reduce the capacity of the oocyte to participate in fertilization and subsequently support embryonic development [1, 2, 4, 23, 24]. Significant biochemical abnormalities result in the reduced activity of critical proteins [e.g., maturation-promoting factor (MPF) and mitogen-activating protein kinase (MAPK)], impaired Ca^{2+} homeostasis, increased autophagy-related activity, mitochondrial dysfunction and disruptions to cell-cycle and stress response pathways [2, 15, 16, 24–28].

In this context, recent work in the mouse has shown that the onset of post-ovulatory oocyte aging is precipitated by an imbalance in cellular antioxidants and ROS levels [4]. Furthermore, in vitro studies have shown that mouse oocytes can be induced to display an aging phenotype following exposure to electrophilic aldehydes such as acrolein and 4-hydroxynonenal (4HNE) resulting in a dramatic decline in the fertilizability of these cells [2, 28]. Such consequences are not surprising, considering the integral contribution electrophilic aldehydes play in a number of oxidative stress-associated diseases, including: diabetes, cancer, atherosclerosis, acute lung injury, chronic alcohol exposure, and in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease (reviewed in [29–31]). In addition, this model may also account for reduced pregnancy rates and higher incidences of cytogenetic abnormalities in humans associated with the use of in vitro aged oocytes following 'rescue ICSI' strategies [32, 33]. Although not specifically reviewed here, it is also important to note that oocytes from individuals of increasing maternal age also display many of the characteristic hallmarks of oxidative stress and the corresponding loss in fertility of those which undergo post ovulatory aging (for review, see [34–38]).

In the search for strategies to prevent oxidative threat to female fertility, sirtuins [silent information regulator 2 (Sir2) proteins] and antioxidant supplementation have emerged as candidates to modulate oxidative assault. Sirtuins appear to play a role in sensing and modulating cellular redox status as well as directly deacetylating key proteins involved in the cellular stress response, thus having been shown to provide protective effects in cells and tissues exposed to oxidative stressors in vitro and in vivo [39, 40]. Supplementation of antioxidants, such as melatonin or caffeine, also appears to provide a valuable, yet temporary, solution to oxidative insult in oocytes. However, while such interventions can delay oocyte aging, they do not appear to be able to prevent this phenomenon entirely [2, 4, 23, 27, 41]. While it is clear that ROS can precipitate a number of negative consequences within oocytes, other factors, such as in vitro handling techniques

and oocyte age (as a result of maternal age), have also been correlated with degradation of oocyte quality. This not only encompasses the consequences listed previously, but also manifests in a reduction in abundance of maternal effect proteins, loss of RNA-binding proteins, critical alterations of pericentromeric proteins, aneuploidy, and epigenetic changes inherited by the derivative generation(s) [42–44]. Undeniably, it is only the act of fertilization that can effectively truncate the post-ovulatory aging phenotype and the inevitable induction of apoptosis [4, 15]. Thus, understanding the precise biochemical and physiological modifications that are initiated upon gamete fusion may hold the key to our attempts to prolong the viability of oocytes and the developmental competence of early embryos.

The role of ions as post-fertilization signaling molecule

At the moment of fertilization, the spermatozoon is responsible for activating embryonic development by virtue of its ability to promote a transient elevation and subsequent oscillating waves of intracellular Ca^{2+} levels within the oocyte [5, 6]. Such events release the oocyte from its MII stage arrest and drive it toward embryogenesis via stimulation of meiotic resumption, cortical granule exocytosis, decondensation of the sperm nucleus, recruitment of maternal mRNAs, and pronuclear development [45].

In mammals, the initial burst of Ca^{2+} is of a longer duration and amplitude than that of the subsequent transients [8, 46]. More importantly, while Ca^{2+} has distinctive short-term effects on the initiation and completion of oocyte activation events, it has also been implicated in the downstream events encompassed by peri-implantation development and gene expression [47–49]. Support for the central role of Ca^{2+} in stimulating embryonic development rests with a series of elegant studies incorporating intracellular Ca^{2+} chelating agents [such as 1,2-bis(o-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid (BAPTA-BA)], which prevents cellular depolarization, metaphase II exit, cortical granule exocytosis, and pronuclear formation [50, 51]. Additional supporting evidence has been secured from experiments involving the judicious use of chemicals such as strontium chloride ($SrCl_2$) and ethanol (EtOH) to artificially stimulate an increase in intracellular Ca^{2+} concentration, and thus drive the chemical or parthenogenetic activation of an oocyte in the absence of a fertilizing spermatozoon [15, 52–55]. The utility of such an approach is recognized by the routine use of artificial activators, together with a spermatozoon, as a supplement in ART settings when the male gamete is unable to activate the oocyte [15, 52–55]. Despite recognition of the importance of Ca^{2+} in these events, the specific sperm factor(s) and signal transduction pathways responsible for triggering its initial release after sperm–oocyte fusion remain unclear and the subject of considerable controversy [13, 14, 56, 57].

What is clear, is that at the moment of fertilization, the hydrolysis of the phosphatidylinositol 4,5-bisphosphate (PIP_2) phospholipid is initiated resulting in the release of cleaved inositol trisphosphate (IP_3) and diacylglycerol (DAG), the former of which binds to IP_3 receptors (IP_3Rs) located in the endoplasmic reticulum (ER), thereby stimulating the release of stored Ca^{2+} [58, 59]. Indeed, the injection of native IP_3 or IP_3 analogs is sufficient to induce Ca^{2+} release in mammalian oocytes [8, 60]. Conversely, inhibitory antibodies and pharmacological reagents that prevent IP_3 binding to IP_3Rs are able to elicit a potent suppression of fertilization induced Ca^{2+} oscillations and subsequently arrest fertilization and downstream embryonic development [58, 61]. The factor(s) that link these signaling phenomena to upstream sperm fusion may be expected to take the form of either oolemmal receptor(s) and/or soluble factor(s) delivered by the fertilizing spermatozoon [62, 63]. Indeed, numerous hypotheses have been put forward to account for the origin of the signal that stimulates the early events of fertilization [13, 14, 56].

Presently, the most widely accepted model centers on sperm specific factor(s) (SSF) that rouse the oocyte by promoting the initial surge in $[Ca^{2+}]_i$. Two purported sperm borne factors that putatively fulfill this role are phospholipase C zeta (PLCζ) and post-acrosomal WW-domain binding protein (PAWP) (Fig. 1) [8, 64, 65]. Indeed, experiments in which either purified PLCζ or PAWP has been injected into an MII stage oocyte have successfully stimulated the production of Ca^{2+} transients that are akin to those that ensue after sperm fusion [60, 64]. Since their initial identification however, numerous research groups have independently reported evidence conferring support for the role of PLCζ in oocyte activation and release of stored Ca^{2+} [8, 66–68]. In this regard, a considerable evidence base has now been established supporting a strong correlation between abnormalities in the structure, expression, and localization pattern of human PLCζ with that of oocyte activation deficiency (OAD) and total fertilisation failure (TFF) [69]. In contrast, since its original identification in 2007, independent research groups have yet to corroborate the ability of PAWP to successfully activate oocytes and/or induce Ca^{2+} oscillations (reviewed in [13]). Indeed, with the generation of a PAWP knockout mouse, it now appears that depletion of PAWP does not elicit the anticipated quantitative change in Ca^{2+} oscillations or in the subsequent rates of embryo development [57].

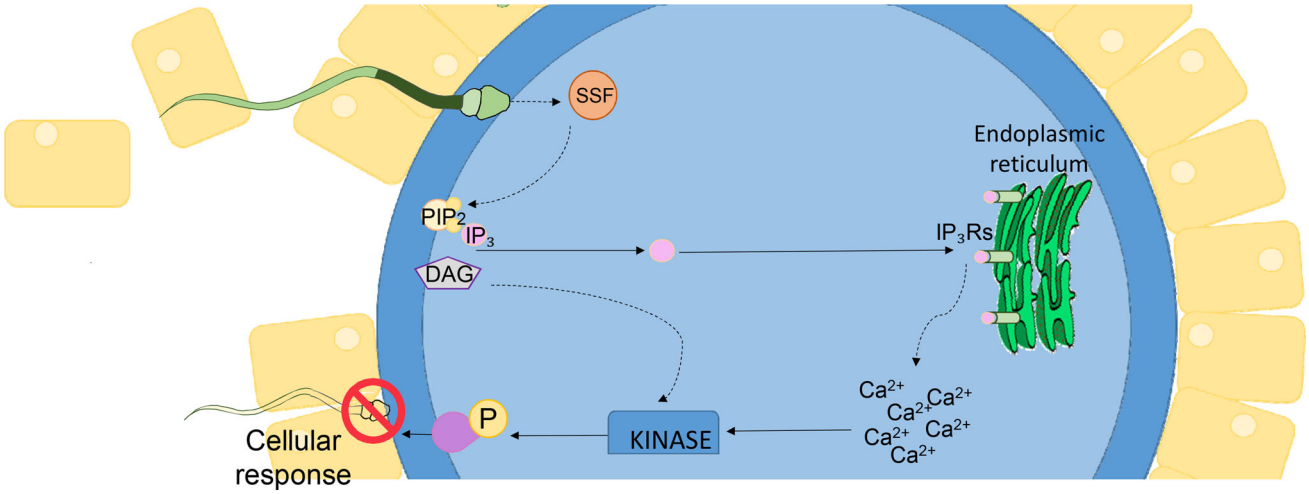

Fig. 1 Fertilization from fusion to activation. The signaling phenomena necessary for oocyte activation and embryonic development encompasses the hydrolysis of the phosphatidylinositol 4,5-bisphosphate (PIP_2) phospholipid anchored in the plasma membrane of the oocyte triggering the subsequent release of cleaved inositol trisphosphate (IP_3) and diacylglycerol (DAG). The released IP_3 is then able to bind IP_3 receptors (IP_3Rs) embedded within the endoplasmic reticulum (ER) and stimulate the release of stored Ca^{2+} to initiate the cellular responses required for oocyte activation. Among the putative sperm-specific factor(s) (SSF) that link sperm fusion to PIP_2 hydrolysis, phospholipase C zeta (PLCζ) and/or post-acrosomal WW-domain binding protein (PAWP) have emerged as key contenders (Adapted from [13])

Ultimately, the definitive identification of the key sperm factor(s) and their mode of action may be crucial for the development of therapeutic intervention strategies to extend the viability of the oocyte, and may also hold value as a prognostic biomarker for the diagnosis of male factor infertility [56, 68, 70, 71]. In this context, strong correlations have been drawn between PLCζ and PAWP protein levels and the success of assisted reproductive cycles, with many instances of infertile human spermatozoa having been found to be deficient in their ability to stimulate the Ca^{2+} oscillations necessary for successful fertilization [72–75]. This work would also benefit from further analysis of the putative synergistic roles that have recently been assigned to dynamic fluxes in alternative ions such as zinc (Zn^{2+}) [76–78]. While still in their relative infancy, the study of the newly coined 'zinc-sparks' has revealed a striking redistribution of Zn^{2+} loaded vesicles immediately at fertilization and demonstrated an inverse relationship between declining Zn^{2+} levels and the all-important increase in Ca^{2+} that is required for successful fertilization, oocyte activation and egg–embryo transition [76–78].

Indeed, during the final hours of meiotic maturation, the mouse oocyte accumulates an impressive twenty billion Zn^{2+} ions (representing an approximate 50 % increase in total Zn^{2+} content) [79, 80], via the two maternally derived and cortically distributed zinc transporters, ZIP6 and ZIP10 [77, 80–82]. Despite this, it is now widely accepted that release from MII arrest requires a dramatic decrease in intracellular Zn^{2+} content. Thus, the act of fertilization must trigger the coordinated release of billions of Zn^{2+} ions, and appears to do so via a novel exocytotic event referred to as a 'zinc-spark.' This process is necessary to re-establish cell cycle progression, oocyte activation and induce the egg-to-embryo transition [76–78, 81]. Accordingly, zinc-sparks appear to be evolutionarily conserved in all mammalian species studied to date, including humans, rodents, and nonhuman primates [77, 79]. The importance of Zn^{2+} homeostasis for oocyte biology is further emphasized by recent studies in which the sequestration of zinc using the heavy metal chelator N,N,N',N'-tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN) [83], or the targeted disruption of Zn^{2+} transporters (ZIP6 and ZIP10), both led to immature telophase I-like cell cycle arrest; a response that could be reversed by Zn^{2+} supplementation [77]. Similarly, oocyte zinc-spark profiles have been positively correlated with mouse embryonic development and embryo quality. Thus, those oocytes that released higher concentrations of Zn^{2+} immediately following fertilization displayed the greatest embryonic development potential [84]. In view of such information, zinc-spark profiles hold considerable promise as a novel extracellular physicochemical biomarker of embryonic developmental potential [84].

Despite the clear biological and clinical importance of Ca^{2+}, and now Zn^{2+}, a rapid induction of transient fluxes in the intracellular concentration of either ion, would not in themselves be sufficient to support conception. Rather, it is likely that these ion(s) act in either an indirect and/or direct manner to promote widespread post-translational modifications (PTMs) across a suite of key enzymes (e.g., protein kinases (PK), phosphatases, and acetyltransferases) that themselves are responsible for promoting the changes in

cellular physiology necessary for oocyte activation and embryonic development [85].

Phosphorylation and fertilization

Protein post-translational modifications (PTMs) increase the functional diversity of the cellular proteome via the covalent addition of functional groups, proteolytic cleavage of regulatory subunits or degradation of entire proteins. These chemical modifications influence almost all aspects of normal cell biology and pathogenesis [86], and their requirement during fertilization is driven at least in part, by the unique dependence of the early embryo on a modest store of maternally derived proteins and mRNA to support all of the early events during embryogenesis [87, 88]. Indeed, until recently, the leading paradigms have held that beyond the initial stimulation of oocyte activation, the fertilizing spermatozoon plays limited additional roles in the cleavage stage embryo [88]. Instead, this role is believed to rest predominantly with maternal factors that accumulate during oogenesis and are responsible for directing zygotic genome activation, the cleavage stages of embryogenesis, as well as the establishment of the initial cell lineages [87, 88]. In keeping with this notion, an autonomous transcription program is not established until the 2 (mouse) or 4 cell stages of embryonic development (bovine, ovine, and human) [89–91].

Among a complement of some 300 forms of PTMs, those involving the selective activation and inactivation of substrates via phosphorylation appear to hold a central position in coordinating the regulation of early embryo development (reviewed in [85]). Protein phosphorylation is a dynamic PTM that is mediated by kinases and phosphatases, which selectively phosphorylate and dephosphorylate substrates, respectively. Principally targeting serine, threonine or tyrosine residues, phosphorylation is one of the most important and well-studied PTMs with estimates suggesting that as many as one-third of the proteins in the human proteome are substrates for phosphorylation [92]. Such substrates extend to the fertilized oocyte, where they have been implicated in a diverse suite of physiological responses that encompass: a rapid block to polyspermy, cortical granule exocytosis, polar body extrusion, pronuclear development, plasma membrane reorganization, recruitment of maternal mRNAs, DNA repair, and resumption of the cell cycle (Fig. 2, Table 1).

The vital role of protein kinases in oocyte activation has been eloquently described in *Drosophila melanogaster*, where 311 proteins were shown to exhibit a change in phosphorylation status between mature and activated oocytes [93], suggesting that phosphorylation might simultaneously and rapidly modulate the activity of many proteins. Chief among these protein targets were those integral to Ca^{2+} binding, proteolysis, and protein translation, as well as those required for general oocyte activation and post-fertilization developmental stages. A number of kinases and their regulatory subunits were also identified amongst the candidates, including extracellular signal-regulated kinases (ERK) [also known as MAPK (mitogen-activated protein kinases)] and A-kinase anchor protein 200 (a regulatory subunit required for PKA localization) [93].

Like that of *D. melanogaster*, the mouse oocyte experiences a swift and dramatic alteration to its global phosphorylation status following both in vivo and in vitro activation [94]. Of particular interest, protein kinase C (PKC) activity has been linked to fertilization-induced Ca^{2+} oscillations (Table 1) using fluorescent C-kinase activity reporter (CKAR) probes in tandem with the selective PKC inhibitor, Gö6976. Interestingly, in vitro manipulation of PKC has revealed an additional role for this enzyme in the stimulation and maintenance of the Ca^{2+} oscillations that drive its activation, suggesting that it forms part of an important regulatory feedback loop [95]. As an extension of this model, Gonzalez-Garcia et al. [96] also showed that PKC-induced phosphorylation outlasts each Ca^{2+} transient, thus raising the possibility that it has a prolonged influence over such downstream events as pronuclear formation, spindle dynamics, cytoskeletal reorganization, cell cycle resumption, and DNA repair [97, 98]. In addition to PKC, oscillating Ca^{2+} has also been implicated in stimulation of calmodulin-dependent protein kinase II (CAMII), a response that triggers the phosphorylation and systematic degradation of non-essential proteins through ubiquitination [85, 99], resumption of meiosis and cortical granule exocytosis [100] (Table 1). Accordingly, CAMII activity was also found to spike almost immediately prior to, and remain elevated following, the extrusion of the second polar body (1.5 h after insemination of oocytes with spermatozoa) [100]. In a similar manner, additional kinases, such as myosin light chain kinases (MLCK), zipper-interacting protein kinase (ZIP), and Rho-kinase (ROCK), have each been implicated as playing integral roles in cytoskeletal arrangement during the fertilization cascade (Table 1). Such activity appears to be mediated, at least in part, via a conserved mechanism involving the phosphorylation of serine 19 (Ser19) on myosin regulatory light chains [101]. This phosphorylation event stimulates actin-mediated ATPase activity and the assembly of myosin II into filaments, thereby promoting cytoskeletal remodeling, cortical granule exocytosis, cytokinesis, polar body extrusion, and cleavage [85, 101]. In support of such functions, it has been demonstrated that selective pharmacological inhibition of MLCK, ZIP, and ROCK (by blebbistatin, ML-7 or Y-27632, respectively) is able to ablate the formation of the second polar body and the correct spindle rotation required for normal cytokinesis

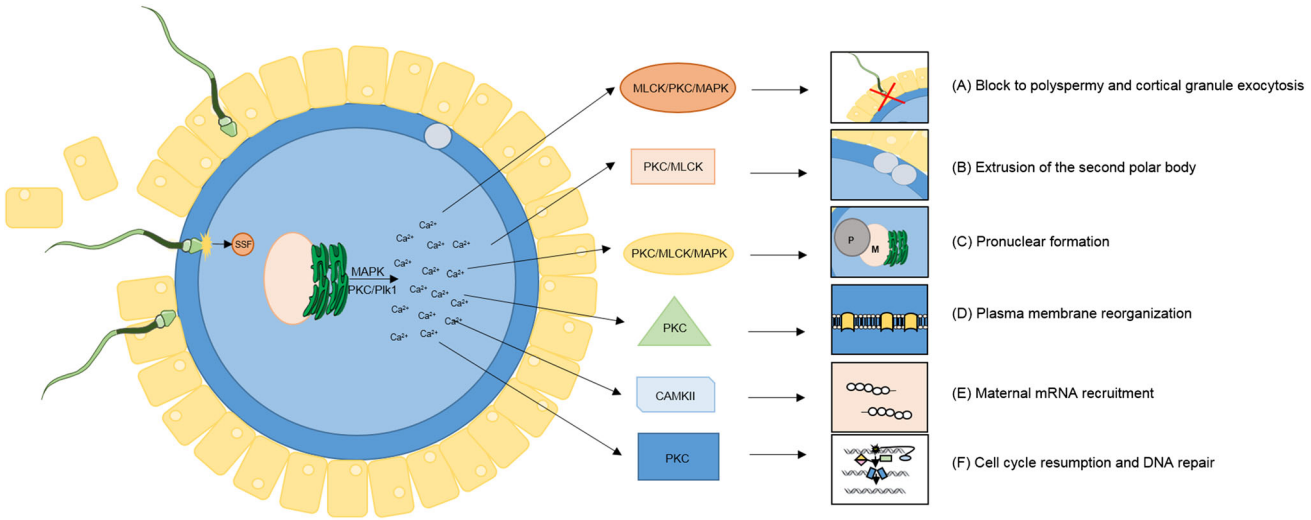

Fig. 2 Protein kinases are intimately tied to each essential event of oocyte activation. Protein kinases and phosphorylation events have been directly implicated in transducing the calcium (Ca^{2+}) signal into many of the necessary activation events required for successful fertilization. Such events encompass a swift block to polyspermy, cortical granule exocytosis, polar body extrusion, pronuclear development, plasma membrane reorganization, recruitment of maternal mRNAs, DNA repair and resumption of the cell cycle (See Table 1) (Adapted from [85])

[102–107]. The role of myosin phosphorylation during fertilization is further underscored by studies in which mouse oocytes were microinjected with nonphosphorylatable myosin regulatory light chain peptides [108]. Such a strategy has been shown to effectively block sperm incorporation cone disassembly and obstruct cell cycle progression with pronounced consequences for successful fertilization [108].

Extending beyond the induction of fertilization, anomalous kinase activity and thus the fidelity of downstream phosphorylation events have been implicated in the etiology of infertility as well as gross phenotypic and developmental abnormalities in offspring [108–110]. Of particular concern, disruption of global phosphorylation by pharmacological or antibody inhibition has been shown to lead to abnormal cortical granule exocytosis, redistribution of the ER and IP_3R1s, disruption of the second polar body formation and extrusion, as well as aberrant cytoskeletal reorganization and cleavage during embryogenesis [100]. For instance, mutation of MAPK signaling pathways abrogates normal IP_3R1 phosphorylation required for the optimal release of Ca^{2+} ions at fertilization; those oocytes deprived of the MAPK signaling pathway during maturation fail to mount normal Ca^{2+} oscillations and show compromised IP_3R1 function leading to compromised or arrested development [111]. Not surprisingly, phosphorylation of IP_3R1 by Polo-like kinase1 (PLK1) also appears to underlie the spatial and temporal regulation of intracellular Ca^{2+} signals required for oocyte maturation. In fact, PLK1 has been shown to co-localize with MAPK and its activity is reduced in the absence of MAPK/ERK activity [112] with devastating consequences for fertilization. Similarly, inhibition of CAM kinase II by pharmacological means [myristoylated-AIP (autocamtide-2-related inhibitory peptide)] disrupted the inactivation of MPF (maturing promoting factor), preventing cell cycle resumption and cortical granule exocytosis in both fertilized and ethanol-activated oocytes [100].

Collectively, these data highlight the importance of phosphorylation cascades in several pivotal aspects of oocyte activation. First, this form of PTM directly affects the activity of a diverse suite of protein targets with dominant roles in transducing the initial Ca^{2+} signal into embryonic activation. Second, even subtle disruptions to these integral pathways can cause dramatic and irreversible consequences for future offspring. Moreover, when these disruptions occur on a broad scale, they can elicit gross biochemical, phenotypic and genomic abnormalities culminating in arrested embryonic development. Such a situation arises, at least in part, because a majority of the enzymes involved in intrinsic cellular repair pathways, such as base excision repair (BER) and homologous recombination (HR), require activation via phosphorylation (or other forms of PTMs) prior to being able to engage in the detection and mitigation of DNA damage (reviewed in [113–115]).

DNA repair within the oocyte

At the moment of fertilization, the majority of oocyte activation events either occur in parallel with, or in quick succession after, the initiation of Ca^{2+} oscillations.

Table 1 Phosphorylation directs key physiological events required for successful fertilization and early embryonic development. Widespread post-translational modifications, in particular phosphorylation has been implicated in directing a diverse suite of physiological responses required for oocyte activation and early embryonic development. A remarkable degree of redundancy exists between kinases at fertilization suggesting the highly integrated nature of these processes for the successful activation of development. A number of key kinases, their regulators, and proposed functions are listed in this table

Kinase	Regulated by	Proposed function(s)	References
Conventional protein kinase C (cPKC)	Intracellular Ca^{2+} DAG Phosphatidylserine	Stimulation/maintenance of Ca^{2+} oscillations Extrusion of the second PB Cell cycle resumption/MII exit PN formation Spindle dynamics/organization Cytoskeletal reorganization DNA repair Block to polyspermy Cortical granule translocation/exocytosis Differential activation of kinases	[85, 95–98, 172–174]
Novel protein kinase C (nPKC)	DAG Phosphatidylserine	Meiotic spindle dynamics Formation/extrusion of the second PN Cortical granule translocation	[95, 175, 176]
Atypical protein kinase C (aPKC)	Phosphatidylserine Negatively charged phospholipids	Regulation of nuclear activity Cortical granule exocytosis	[177]
Calmodulin-dependent protein kinase II (CAMKII)	Intracellular Ca^{2+} bound to calmodulin (CAM)	Protein degradation by ubiquitination Decrease in MPF/resumption of meiosis Cortical granule translocation/exocytosis Maternal mRNA recruitment MII exit Cyclin B1 and securin destruction Decreases in the activity of MAPK Second PB extrusion Formation of maternal PN Phosphorylation of IP_3R1s Regulation of apoptosis Sister chromatid segregation/ decondensation	[85, 99, 174, 178–182]
Polo-like kinase1 (PLK1)	Intracellular Ca^{2+}	Redistribution of the ER Phosphorylation of IP_3R1s Regulation of Ca^{2+} Enhance receptor function on ER Removing an APC/C inhibitor	[85, 112, 183, 184]
Myosin light chain kinases (MLCK)	Intracellular Ca^{2+} phosphorylation of myosin II	Block to polyspermy PN formation Cytoskeletal remodeling PB formation/extrusion Cleavage/cytokinesis Redistribution of the ER and IP_3R1s Calcium oscillations Regulator of myosin II in non-muscle cells Spindle rotation Morula-to-blastocyst transition	[85, 102–107, 185]

Table 1 continued

Kinase	Regulated by	Proposed function(s)	References
Mitogen-activating protein kinase (MAPK) and Extracellular signal-regulated kinases (ERK)	Intracellular Ca^{2+} CSF activity	Activation and regulation of Ca^{2+} release Formation of PN Disintegration of the pronuclear envelope Cortical re-organization prior to fertilization Translocation of the meiotic spindle Redistribution of the ER and IP_3R1s Formation of ER and IP_3R1 cortical clusters phosphorylation of IP_3R1	[85, 93, 102, 106, 111, 186, 187]
M-phase kinases or Maturation promoting factor (MPF)	Intracellular Ca^{2+} ATP Cyclin	Stimulation/maintenance of Ca^{2+} oscillations promoting resumption of meiosis and cell-cycle transitions Nuclear membrane breakdown	[85, 186]
Rho-kinase (ROCK)	Intracellular Ca^{2+}	Rho-furrowing phase of cytokinesis Spindle rotation Polar body formation Phosphorylation of IP_3R1 Cytoskeletal rearrangement Ooplasmic segregation Spindle rotation	[86, 112, 186, 188–190, 107]
Zipper-interacting protein kinase (ZIP kinase)	Intracellular Ca^{2+}	Regulator of myosin II required for cytokinesis	[101]
Protein kinase A (PKA)	Intracellular Ca^{2+}	Phosphorylate IP_3R1	[175, 191]
Protein kinase G (PKG)	Intracellular Ca^{2+}	Phosphorylate IP_3R1	[192]
Tyrosine kinases (FYN and LYN)	Intracellular Ca^{2+}	Phosphorylate IP_3R1	[193, 194]
Protein kinase B (PKB)	Intracellular Ca^{2+}	Phosphorylate IP_3R1	[195]

However, at the time of pronuclear formation, the conceptus experiences a transient arrest of Ca^{2+} oscillations [7]. Recent evidence has raised the possibility that this phenomenon is staged to allow a critical round of DNA repair to occur prior to DNA replication during the first mitotic division [12]. In addition, this temporary suspension of Ca^{2+} oscillations enables the reconfiguration of the sperm chromatin prior to pronuclear formation and syngamy [116]. However, the capacity of preimplantation stage embryos to repair damaged DNA remains to be fully characterized, and it seems that phosphorylation of existing repair proteins and maternal (and more even recently paternal) mRNAs capable of damage detection and cell cycle control are likely responsible for directing DNA repair [117–121]. Fittingly, the effects of paternal miRNAs seem to be mediated, at least in part, by post-transcriptional regulation over maternal and early zygotic mRNAs (e.g., miRNA mediated mRNA stability control; refer to section 'Epigenetics and DNA repair') [117, 122]. The quality of the paternal and maternal genetic contributions at fertilization greatly influences the developmental competency of the derivative organism [123]. It follows that the absence of efficient DNA repair at this critical developmental phase can either result in complete embryonic arrest or poor embryo development associated with increased risks of immunodeficiency, neurological disorders, and cancer within offspring and a concomitant reduction in life expectancy [124–127].

While many somatic cells experience a consequential increase in the synthesis of DNA repair enzymes and protective proteins in response to DNA damage [128, 129], the ovulatory stage oocyte is, in contrast, transcriptionally silent and incapable of mounting such a response [130]. Instead, these cells depend on stores of pre-synthesized proteins and/or mRNA transcripts to drive repair pathways during fertilization and early embryo development [131], which additionally may work in combination with paternal epigenetic modulators [118] (see Section 'Epigenetics and DNA repair'). During these transcriptionally inert stages of development, PTM of pre-synthesized and maternally stored repair enzymes are likely to facilitate the activation of the fundamental machinery necessary for mitigating DNA and/or cellular damage. In this context, an impressive array of PTMs (including acetylation, phosphorylation,

methylation, ubiquitination, and sumoylation, as well as histone modification) have been implicated in cellular repair capacity and have been found to dramatically influence the kinetics of DNA repair [23, 127, 132]. Recent research within our own laboratory has provided evidence for the induction of a dramatic series of post-fertilization reparative (and protective) events involved in ensuring oocyte viability prior to embryogenesis [10, 133, 134]. Furthermore, we have also provided evidence for DNA repair and protection pathways with stage-dependent activity in the mammalian oocyte [10, 133, 134]. As a culmination of this work, it has been suggested that oocytes experience accelerated repair of oxidative DNA damage following fertilization driven by post-translational modifications of proteins that participate in the BER pathway [10] (Fig. 3a). This pathway, which includes the key elements of oxoguanine glycosylase (OGG1), apurinic/apyrimidinic endonuclease (APE1), and X-ray repair cross complementing protein 1 (XRCC1), is capable of repairing the common oxidative DNA base adduct, 8-hydroxyguanosine.

While the BER pathway is considered the primary orchestrator of oxidative damage repair in the zygotic genome, a host of other enzymes have the capacity to contribute to these and other repair processes [134]. A recent proteomic comparison of murine MII oocytes and zygotes identified a total of 53, and in primate oocytes, approximately 37 proteins involved in DNA damage and DNA repair related processes [121, 130]. In the MII oocyte, enzymes required for nucleotide excision repair (NER), single strand break repair (SSB), double strand break repair (DSB), and BER are all highly expressed [121, 130], thus highlighting their potential for activation and use immediately following fertilization. In the same manner, a proteomic analysis of high- and low-quality oocytes in a porcine model indicated a positive correlation between the abundance of DNA repair proteins and the quality of the oocyte [135]. Microarray and bioinformatic approaches have also confirmed the expression of a similar profile of proteins in human oocytes and early embryos [136].

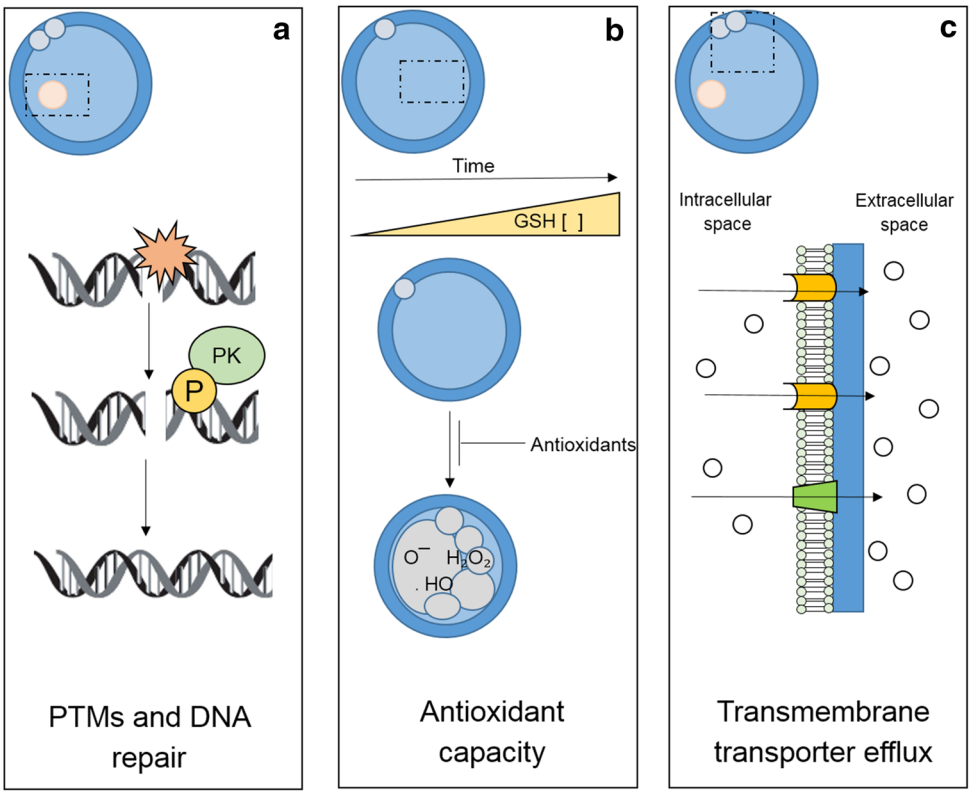

Fig. 3 Fertilization induces a dynamic suite of protective and reparative events to ensure successful egg–embryo transition. These include: **a** post-translational modifications (PTMs) of pre-synthesized maternally derived repair enzymes that provide the fundamental machinery necessary for mitigating DNA and/or cellular damage in the early embryo; **b** significant alterations in glutathione (GSH) and glutathione peroxidase activity, thus truncating the effects of post-ovulatory oocyte aging and defending the embryo against the accumulation of oxidative DNA damage; and **c** a complex assortment of transmembrane transporter molecules implicated in facilitating the removal of genotoxic agents from the cytosol (Adapted from [2, 10, 126, 127])

In addition to direct DNA repair, the oocyte is endowed with a host of alternative protective systems that may serve as the first line of defense against various forms of cellular damage including oxidative insult [10, 126, 127, 137]. In some instances, these protective systems are differentially expressed and/or activated in peri- and post-fertilized oocytes, generally rendering these cells less susceptible after fertilization has commenced. Examples include transmembrane transporter proteins involved in drug exclusion and antioxidant defenses that act to resolve oxidative DNA base adducts and prevent protein alkylation and mitochondrial disruption [10, 131, 133, 134]. In this context, accumulating evidence indicates that immediately prior to fertilization, concentrations of the antioxidant glutathione (GSH) are significantly increased in the oocyte, and remain elevated during the zygote stage, before precipitously falling by the 2-cell embryo stage [138]. Consistent with these data, it has also been shown that glutathione peroxidase activity is increased in zygotes compared to MII stage oocytes, thus providing additional protection to the zygote against hydrogen peroxide-induced DNA strand breakage [10; Fig. 3b] in conjunction with its essential role in paternal pronucleus formation and successful preimplantation development [139].

Epigenetics and DNA repair

Perhaps not surprisingly, embryonic quality and the effective maintenance of genomic integrity are influenced by the epigenetic signatures of both parental gametes [140]. Indeed, it appears that the correct establishment of epigenetic modifications and subsequent chromatin scaffolding are crucial steps in regulating the fidelity of the first mitotic cleavages with their absence leading to pronounced consequences for the development of a preimplantation embryo [127]. In this context, it has been shown that the inhibition of H3K4 demethylation can inhibit cleavage to the 4-cell stage embryo [127], while the inactivation of histone lysine methyltransferase (KMT5A) induces early embryonic lethality prior to the 8-cell stage [141]. Similarly, the deletion of Jumonji C domain-containing demethylase (JMJD2C) arrests embryo development prior to the formation of a blastocyst [142]. Recent evidence also suggests that the propensity of epigenetic modifications to modulate contact between nucleosomes and chromatin may serve to influence the compaction and/or relaxation state of the DNA fiber [143, 127]. In this capacity, epigenetic modifications may not only 'open' and 'close' regions of the DNA for transcription, but they may also act as important molecular gatekeepers during DNA decondensation and repair by providing the requisite enzymes with appropriate access to the DNA template [143–145]. In this respect, an enrichment of proteins responsible for epigenetic modification and chromatin remodeling (e.g., SMARCA5, CHD3, and CHD4) has been found in the mouse MII oocyte and early embryo, a particularly important consideration given the transcriptionally inert nature of immature oocytes prior to fertilization [123]. In further support of this notion, a dramatic loss in DNA demethylation [as evidenced by a reduction in 5-methylcytosine (5mC) content] has been recorded shortly after the protamine-histone exchange that occurs within the paternal genome following fertilization [146, 147]. This demethylation phenomenon appears to be closely associated with the appearance of DNA DSBs (γH2A.X) and DNA repair markers [Poly(ADP-Ribose) Polymerase 1 (PARP-1)] [148]. Furthermore, the distinctive co-localization of γH2A.X foci formation with the sites of demethylation within the paternal pronucleus during the pre-replicative stages of development, has led some to postulate that DNA demethylation may be regulated by indirect DNA repair-induced mechanisms such as the BER or NER pathways [149]. Such a model is consistent with the fact that the paternal gamete must decondense before syngamy [116], and that the maternal gamete is responsible for ensuring the genetic integrity of both nuclear contributions [87, 88].

Interest is also beginning to focus on the epigenetic regulation imposed by the fertilizing spermatozoon [150, 151]. In this regard, it is now recognized that, in addition to delivering the signal(s) that initiate the fertilization cascade, the male gamete may also contribute developmentally important epigenetic modulators (e.g., DNA methylation, sperm-specific histones, and other chromatin-associated proteins), as well as a number of small noncoding RNAs (sncRNAs), to the oocyte during fertilization that may all participate in successful embryogenesis [122, 151–153]. A strong case for this form of regulation has been mounted on the basis of experiments involving the use of conditional germline specific, *Dicer* and *Drosha* knockout mouse models. These studies have revealed that an embryos developmental potential, transcriptomic homeostasis and early zygotic gene activation are each dependent on paternally derived sncRNA cargo [117, 122, 151]. It has also been suggested that a specific sub-class of sncRNAs, known as the small interfering RNAs (siRNAs), may participate in key developmental processes encompassing pronuclear formation, DNA repair, orchestrating oocyte activation, the transition from maternal to embryonic gene control, and the establishment of imprints in early embryos [154]. Such findings offer many exciting avenues for future research, not the least of which will be to determine how epigenetic mechanisms of regulation are seamlessly integrated with maternally mediated PTM of signaling pathways to support early embryonic development.

Additional protective mechanisms of the oocyte and early embryo

In recent years, a variety of additional mechanisms have been identified that may prevent the propagation of damage in the fertilized oocyte. In marine invertebrates as in some mammalian species, post-fertilization activation has been implicated in promoting the synthesis of transmembrane transporter molecules, such as the ABCB protein, P-glycoprotein (PGP), and an ABCC protein similar to the multidrug resistant (MDR)-associated protein (MRP)-like transporter [154]. Though poorly understood, these proteins appear to be trafficked to the plasma membrane where they become functionally active and thereafter increase the bi-directional transport capacity of the cell [133, 134, 154]. Such proteins have been implicated in the transport of genotoxic agents from the intracellular environment and away from the vulnerable genomic material [133, 134, 155], as well as shuttling hormones and amino acids from the surrounding environment to the zygote to facilitate growth and development [156]. In somatic and cancer cell models, a similar complement of transmembrane transporter proteins has been implicated in multidrug resistance [157]. In these cells, the transport activity of the proteins appears to be intimately tied to their activation by post-translational phosphorylation events driven by Ca^{2+} and the serine/threonine kinases of PKC and/or PKA [133, 134, 158–160]. These results are particularly interesting given the notable increase in PKC activity that flows from the elevated levels of intracellular Ca^{2+} as well as a secondary stimulus of DAG, which are present at the time of fertilization. Taken together, it is tempting to speculate that PKC may hold a key role in the increased activation of transmembrane transporters to help protect mammalian oocytes and early embryos (Fig. 3c).

If this hypothesis was correct, it may afford unique opportunities to increase the protection of maturing oocytes with important implications for assisted reproductive strategies. In this regard, the 'drugability' of kinases [161] and their protein targets could provide avenues for the improvement of oocyte and early embryonic quality in vitro. The merits of this approach have been emphasized by recent proof-of-concept studies. For instance, artificial upregulation of transmembrane transporter molecules in bovine and porcine oocytes has been shown to have a dramatic positive impact on the post-cryopreservation viability of late stage embryos [162, 163]. In addition, recent literature indicates that numerous pharmacological (bovine embryos: rifampicin and forskolin) and hormonal (porcine GV stage oocytes: progesterone) signals can significantly elevate membrane transporter protein expression and/or activity, respectively [162, 163]. This paradigm provides an exciting prospect in clinical IVF settings whereby culture medium could be supplemented with compounds to increase the efflux activity of transmembrane transport proteins and thus reduce the intracellular availability of potentially damaging agents. This may also prove to be a valuable mechanism for the protection of the ovary and immature oocytes from both fresh and frozen sources in clinical fertility management.

In the event that the innate prevention and repair systems are inadequate or overwhelmed by a particular insult, mechanisms that are responsible for avoiding the propagation of damage take center stage. This is particularly important when considering the unique role that the oocyte plays in the continuation of a species when, in extreme cases, significant DNA damage could lead to chromosomal loss, translocation or duplication. Cumulatively, such damage could also significantly increase the mutational load borne by the embryo and thus heighten the risk of carcinogenesis in the offspring [164]. Correspondingly, a variety of mechanisms have been identified as being responsible for the removal of severely damaged oocytes or early embryos. These are usually characterized by significant cellular senescence, DNA fragmentation and degradation, and eventually either cell atresia or apoptosis (reviewed in [165–167]). Atresia dominates the mechanisms involved in removal of immature oocytes from the ovarian reservoir and involves ligand–receptor complex systems, including tumor necrosis factor alpha (TNFα), TNF-related apoptosis-inducing ligand (TRAIL or APO-2), Fas ligand, APO-3 ligand, PFG-5 ligand, and associated receptors; on the other hand, mature oocyte and early embryo loss are primarily mediated by Bcl-2 family members, Apaf-1 and caspase activation leading to apoptosis [154, 167–171].

The endowment of such elaborate systems for the detection, prevention, repair, or as a last resort, removal of a compromised cell clearly has evolutionarily benefits for a species. However, the increasing instance of delayed childbirth in our own species means that these mechanisms are working against us, particularly considering the expression of several key repair proteins has been found to be drastically reduced between 'young' and 'aged' oocytes [172] leading to a further potential increase in the demand for assisted reproductive procedures. While the advent of increasingly sophisticated assisted reproductive technologies to bypass such biological obstacles has been remarkably beneficial to many couples wishing to conceive, the reliance on this technology carries the very real risk of exacerbating genetic and cellular damage through gamete handling and culturing procedures in cells that would otherwise fail to participate in fertilization events [36].

Concluding remarks

The process of fertilization elicits a complex suite of biochemical and physiological modifications that prepare the oocyte for sustained embryonic development. These events are intimately tied to a prominent rise in intracellular Ca^{2+} at the moment of fertilization and the concomitant stimulation of an extensive range of oocyte activation events. Chief among these appears to be the activation of kinases that mediate the post-translational modification of a suite of key developmental proteins. It is now known that such events are likely to work in unison with paternally derived small non-coding RNA species, which regulate the stability/translation of the limited pool of maternally stored mRNA transcripts. This review contextualizes the role of fertilization and oocyte activation in extending the viability of the oocyte, initiating a series of biochemical alterations required for embryonic development and highlighting the prominent role that protein PTMs, and in particular phosphorylation, hold in their execution.

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Compliance with ethical standards

Conflict of interest The authors have nothing to declare.

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