A Treatise on Current Advances in Egg Activation Protocols

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Abstract: Ca^{2+}-oscillations pave the way for activation of eggs in many animals that are discussed here. This so called "repetitive" rises is instrumental in embryonic development; the comparative analysis in various animals below illustrates the importance of Ca^{2+} and Sr^{2+} (to some extent), both being divalent cations, in the process of egg development. Only recently it has been found that a sperm-derived factor "phospholipase Cɼ", is the most likely sperm candidate,that is pivotal in the Ca^{2+}-oscillations. Phospholipase Cɼ is capable of converting phosphatidyl inositol 4,5 bis-phosphate into diacylglycerol (DAG) and Inositol 1,4,5-trisphosphate (IP_3). The latter binds to receptors present on the membrane of ER (endoplasmic Reticulum), thereby opening up and aiding in the transport of Ca^{2+} oscillatory rise in the cytosol. This event is instrumental in activating various dormant processes in eggs (described below). Inhibition of the IP_3R1 receptors by function-blocking specific antibodies resulted in lack of embryonic development. At the same time aberrant phopholipase Cɼ expression or the lack of it in males confers sterility for obvious reasons. Artificial activation of eggs is a prerequisite for parthenogenetic activation of eggs in various animals as described below. Egg activation protocols is designed to mimic the $[Ca^{2+}]_i$ responses induced by sperm or to replicate the $[Ca^{2+}]_i$-oscillations which results in inactivation of M-phase kinases leading to release of eggs from MII arrest. . Exposing eggs to ethanol and Ca^{2+}-ionophores viz, A23187 and ionomycin causes a single and sustained $[Ca^{2+}]_I$ increase. It can be concluded fairly certainly that "a single $[Ca^{2+}]_i$ –rise" is insufficient to induce complete cyclin B degradation. Inhibitors of protein synthesis and kinases, like cycloheximide (CHX) and 6-dimethylaminopurine (6-DAMP) are added to the medium for several hours after treatment with the ionophores. In direct comparison to Ca^{2+}ionophores, a brief exposure to $SrCl_2$ or Acetylcholine or thimerosal generates repetitive Ca^{2+} that could last for several hours, resulting in Ca^{2+}-ion signals much closer to the mode of activation by sperm. Moreover administration of agonists of IP_3Rs, such as IP_3 or adenophostin A also induces $[Ca^{2+}]_i$- oscillations. Instead of chemical reagents, multiple $[Ca^{2+}]_i$ –rises is possible if evoked by applying electrical DC pulses in the presence of extracellular Ca^{2+}. Injection of recombinant human PLCζ was tried out in the year 2012 and it induced $[Ca^{2+}]_i$-oscillations in both mouse and human eggs. The production of pure recombinant form of PLCζ has been a goal in recent years and several methods have been employed to ensure the production of the same. Recombinant human PLCζ protein prepared in this way was able to generate Ca^{2+} in a physiological range in mouse and human eggs. Moreover such studies also demonstrated the deleterious effect of mutant PLCζ and the possibility of the same to be overcome by recombinant PLCζleading eggs to develop to blastocyst stage. However this work seems to be extremely encouraging, careful extrapolation may be necessary to arrive at after applying the same in the laboratory and progressing to clinical settings.

Keywords: Egg Activation Protocols, animals, Ca^{2+}-oscillations, egg development.

1. INTRODUCTION

Fertilization marks the initiation of life with subsequent ensuing of embryonic development. Resumption of meiosis from Meiosis II (MII) next takes place, with subsequent changes including (1) exocytosis of cortical granules (2) Extrusion of the second polar body and (3) pronucelar formation [63,75,33,46].

The changes, described above, collectively result in "EGG ACIVATION". In mammalian species studied to present, egg activation involves a rise in intra-cellular level of Ca^{2+}, (henceforth abbreviated as $[Ca^{2+}]_i$) [66]. In these instances egg activation requires an increase in the intra-cellular $[Ca^{2+}]_i$ signal consisting of persistent increases of Ca^{2+} ionic level in the intracellular milieau, (henceforth referred to as $[Ca^{2+}]$-oscillations) [64].
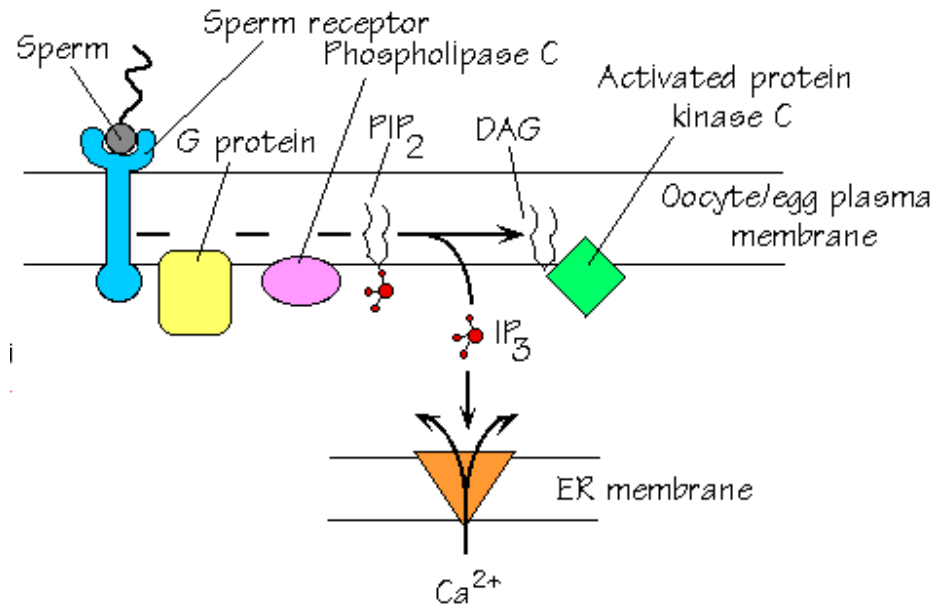

Fig.1: Hypothetical Pathway Illustrating Calcium ion Release: Calcium ion release from ER lumen occurs only when the G protein activates phospholipase C. The G protein is not activated normally but becomes activated only when sperm binds to the receptor present on the plasma membrane of oocyte. As a result of this interaction Phospholipase C converts PIP_2 into DAG and IP_3. The latter binds to the receptor on the ER membrane of oocytes as a result of which the channel opens up leading to oscillatory rise of Ca^{2+} in the cytosol, leading to egg activation (Diagram courtesy: Prof. Jeff Hardin, Dept. of Zoology; University of Wisconsin-Madison).
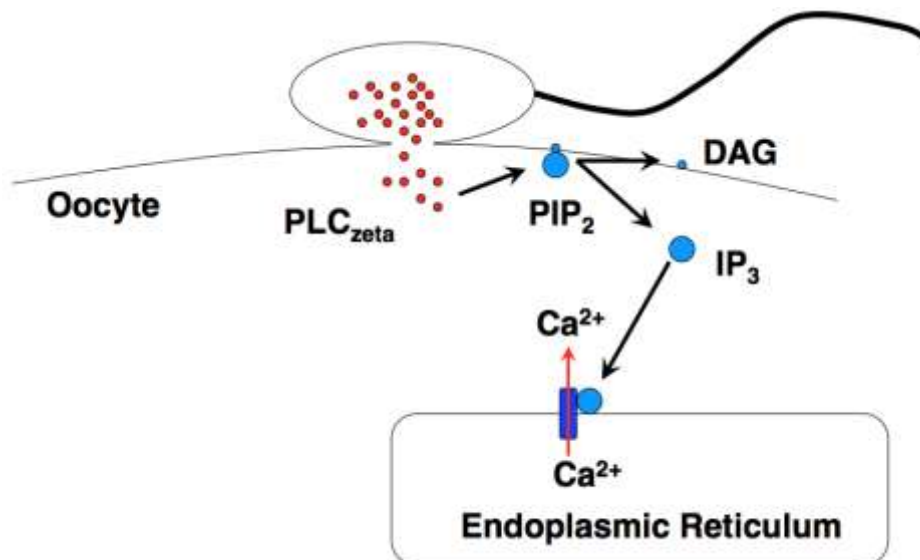

Fig.2: PLCζ Calcium transients in oocytes: An alternative explanation in which Ca^{2+}, is transported to the oocyte cytosol via Ca^{2+} channels present on ER membrane. Here it is shown that sperm membrane fuses with the oocyte membrane leading to release of PLCζ manufactured by the genome of the sperm. The released PLCζ catalyzes the formation of DAG and IP_3 from PIP_2. The IP_3 so formed binds with the membrane channel resulting in a conformational change that allows Ca^{2+} to be transported outside of the ER lumen (Diagram courtesy: Prof. John Parrish, Dept. of Animal Science; University of Wisconsin-Madison).

Only recently it has been found that a sperm derived factor, phospholipase Cʐ, is the most likely sperm candidate responsible for this oscillation[1,5,12,57,64]. PLCs hydrolyze phosphatidyl inositiol, 4,5 bis phosphate (PIP2) into diacyl glycerol (DG) and inositol[7,22,41,77], 1,4,5, tris-phosphate (IP_3)[68,64] (Fig.1). It should be noted that both of these are powerful cell signaling molecules but IP_3 in particular is responsible for triggering intracellular calcium ion rise, by binding to IP_3R (IP_3-Receptors) present mainly on the membrane of Smooth ER (The main calcium ion reservoir) and facilitating transient Ca^{2+}-rise in the cytosol[6,16,21,32]. Proof of this mechanism of egg activation has come from various studies in mammals as sperm extracts were shown to induce IP_3-production[64,27,42,87,68,28,81].

Inhibition of IP_3R1 by function-blocking specific antibodies resulted in the prevention of $Ca2^+$ oscillations, efflux from the ER, and consequently, cessation of embryonic divisions[18,55]. Moreover, patients who fail to express PLCζ in mature sperm, are unable to create the initiation of $[Ca^{2+}]_i$ oscillations, and consequently are sterile, although their sterility may ultimately be bypassed by artificial activation of eggs[19,50,71,86] (Fig.2).

Two important conclusions can be drawn at the outset from the question "What is the requirement of the increase of $[Ca^{2+}]_i$? (1), It is associated with the ability to activate Anaphase Promoting Complex/ Cyclosome (APC/Cyclosome)[56,65,70] and (2) the consequent inactivation of the MPF (Maturation Promoting factor)[29,75,80] (Fig.3). This ensures resumption of meiosis and unfolding of the developmental sequence. Two assisted reproductive techniques that are of paramount importance require artificial stimuli for the activation of the egg in the absence of endogenous activation. The techniques of Somatic Cell Nuclear Transfer (SCNT) and ROSI (Round Spermatid Injection) may be used to achieve these means. Artificial activation of the egg is also a necessity for parthenogenesis of the egg to occur[44,89,76,77,84].
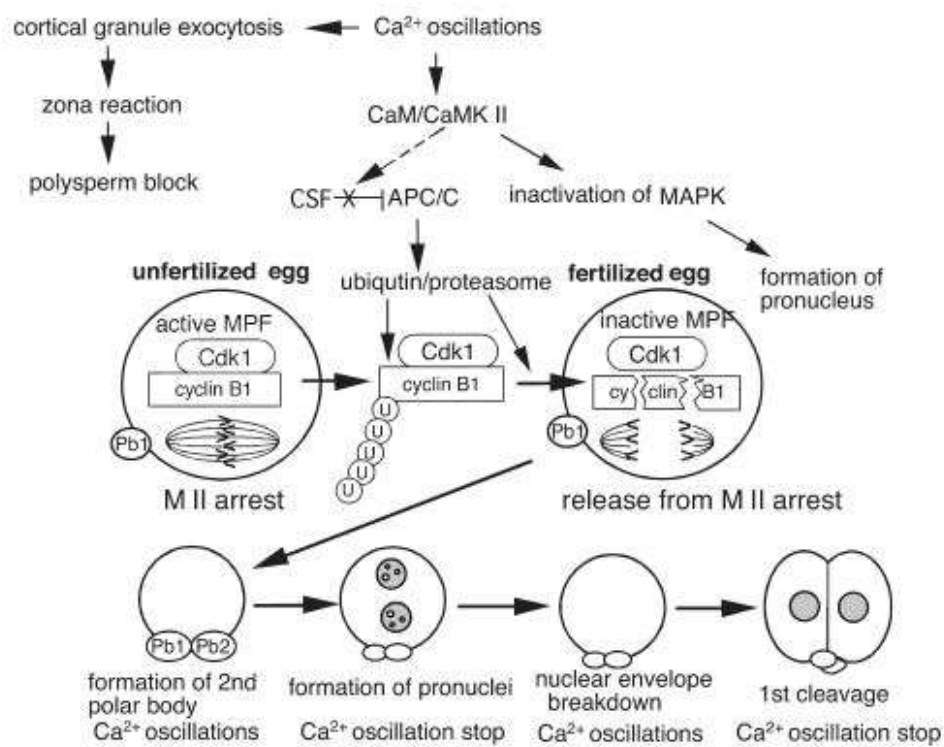

Fig.3: Proposed pathway of release from MII arrest: Ca^{2+} oscillations plays a double role in the theme of Egg Activation. On the one hand it ensures exocytosis of cortical granules leading to zona reaction and a slower block to polyspermy. On the other hand the Ca^{2+} -oscillations is instrumental in the release from MII arrest. An unfertilized egg is arrested in Meiosis II, consists of MPF (Mitosis Promoting Factor), which is made up of Cyclin B1 bound to Cdk1. Ca^{2+} oscillations activates Anaphase Promoting Complex (APC) via Calmodulin (CaM)/CaMK on the one hand and leads to the formation of pronucleus via inactivation of MAPK. The main events which follows release from MII arrest in a fertilized egg is (1) formation of 2nd polar body which happens when Ca^{2+} is there in the cytosol, followed by (2) Formation of pronuclei which occurs in the absence of Ca^{2+}ion. (3) Nuclear membrane breakdown occurs at this stage in the presence of Ca^{2+} followed by the last phase (4) In the absence of Ca^{2+}, the first cleavage occurs (Diagram courtesy: Miyazaki and Ito, 2006[43]).

Current advances in egg activation protocols ensure the production of viable embryos, which includes full term development. But it should be borne in mind that the probability of seeing live offspring is very low. The poor success rate of procuring good quality embryos is certainly due to "Genomic Imprinting" and its "alterations"[88]. So importance is to be given to "improved egg activation protocols and their impacts on molecular mechanisms that control genomic imprinting, methylation and chromosome segregation". Defects in these processes may undermine the development of embryos.

2.   EGG ACTIVTION PROTOCOLS

Several novel approaches have been employed to insure mammalian egg activation, all of which includes exit from MII arrest and entry into the embryonic cell cycle. As earlier indicated, the sperm- induced Ca^{2+}-signals are surely instrumental in initiation and consequent completion of all components involved in egg activation. As a result of this, activation protocols are designed either to mimic the $[Ca^{2+}]_i$ responses induced by the sperm, or to replicate of the $[Ca^{2+}]_i$ oscillations, which results in inactivation of M-phase kinases and consequent exit from MII arrest.

Some methods of artificial activation of eggs are discussed below. Exposing eggs to ethanol and Ca^{2+}-ionophores viz, A23187 and ionomycin which causes a single and sustained $[Ca^{2+}]_i$ increase[13,31]. This signal is enough for induction of the initial steps involved in egg activation including release from the MII arrest[81,33]. The transition from MII stage to interphase is longer in mammals as compared to other vertebrate species. So, during this transition the long-lasting $[Ca^{2+}]_i$ is certainly instrumental in persistent degradation of cyclin B, the regulatory component of MPF. It should be noted that the synthesis of cyclin B is continuous during this period and its persistent degradation is at the heart for inactivation of MPF and MII exit[46]. It can be concluded fairly certainly that "**a single $[Ca^{2+}]_i$ –rise**" is insufficient to induce complete cyclin B degradation[65]. For example premature termination of experimentally imposed $[Ca^{2+}]$ rise results, after an initial period of decline, in a retrieval of MPF activity, which in turn leads to abnormal cell cycle progression and even in some cases, re-arrest of the cell cycle[65].

It is for this specific reason other compounds that sustain low levels of MPF and MAPK activities are combined with ethanol and ionophores to insure transition from MII arrest to interphase[60]. To fulfill this role, inhibitors of protein synthesis and kinases, like cyclohexamide (CHX) and 6-dimethylaminopurine (6-DAMP) are added to the medium for several hours after treatment with the ionophores. It should be noted that these inhibitors could by themselves induce egg activation. However the lack of Ca^{2+}-ion hinders proper gene expression required for development to blastocyst stage[60].

In direct comparison to Ca^{2+}ionophores, a brief exposure to $SrCl_2$, Acetylcholine or thimerosal generates repetitive Ca^{2+} that could last for several hours, resulting in Ca^{2+}-ion signals much closer to the mode of activation by sperm[10,14,87]. Moreover administration of agonists of IP_3Rs, such as IP_3 or adenophostin A also induces $[Ca^{2+}]_i$- oscillations. However, presently these reagents need to be directly injected into the eggs, making them less convenient in situations that demand activation of large number of eggs. Last but not the least, instead of chemical reagents, multiple $[Ca^{2+}]_i$ –rises is possible if evoked by applying electrical DC pulses in the presence of extracellular Ca^{2+}. This treatment results in higher rates of pre-implantation embryonic development to the blastocyst stage[87,52].

Further, it is apparent that repeated application of electrical pulses demands expertise and sophisticated instrumentation, which restricts widespread usage of this ingenious technique. Presuming that most current methods of egg activation result in failure to generate an oscillatory rise in $[Ca^{2+}]_i$, most laboratory applications of the same, requires incorporation of certain drugs which, may not be advisable and species specific drug treatment regimen is the order of the day[53]. This selection should be ideally based on three important generalizations and they are respectively, high rates of egg activation and pre-implantation embryonic development, high rates of survival of the so formed zygotes and lastly, easiness on delivery. Henceforth we discuss, egg activation protocols commonly used for SCNT in varied species, which includes, pigs, cattle and mouse and humans[52].

Egg Activation Procedures in Mouse:

The SCNT procedure in mouse is one of the most refined to date as compared to other mammals. Cloned pups are readily obtained taking the help of different activation methods, which includes $SrCl_2$, ethanol and DC pulses[52]. Of these, the application of $SrCl_2$ is the most widely employed method for generation of SCNT embryos[52]. Although little is known about the channels and mechanistic principles involved in Sr^2 transport, into the cells it is presumed that exposure to $SrCl_2$ induces oscillations by sensitizing IP_3R1, thereby decreasing the effective cellular concentration of IP_3, that is required to gate IP_3R1 and promoting Ca^{2+}-ion release from the ER into the cytosol[15,87,89].

The pattern of oscillations induced resulting from the rise in intracellular $SrCl_2$ is radically different from that induced by sperm, as the duration of each of the rises is characteristically longer than those observed after the sperm unites with the ovum (i.e., during fertilization). Moreover since $SrCl_2$ is not capable of inducing any PLC activation, stimulations of the oscillations is likely to take place without activation of several downstream effectors, generated by these enzymes such as DAG and PKC[26]. It should however be noted that little is known about the impact of the different shape of oscillations and the possible omission of DAG and PKC on Ca^{2+} homeostasis and developmental competence. However there is no semblance of doubt regarding the pivotal role of $SrCl_2$ in initiation, progression and completion of all events associated with the activation of egg. It is to be highlighted that there is no need of any broad-spectrum protein synthesis or kinase inhibitors, in this procedure[38]. Therefore application of $SrCl_2$ is the method of choice for activation of ova for SCNT in mouse.

Common Egg Activation Procedures in Pig and Cattle:

$SrCl_2$-induced oscillations represent a convenient method of activating eggs derived from mice and rats, though it is by no means a universal stimulant. When applied to porcine and bovine eggs with comparable stimuli, failure to induce any parthenogenetic activation without combination with other compounds occurs[36,37,55,85,83,75]. It should be emphasized that monitoring of $[Ca^{2+}/Sr^{2+}]_i$ response which follows stimulation with $SrCl_2$ has not been performed in many of these species. Several studies point to the fact that bovine oocytes fail to mount any type of oscillations when stimulated with 10mM $SrCl_2$, which is the same concentration that is used in activating oocytes of mice and rats[2,4,55].

In view of the above-mentioned reports, combination treatments such as those that combine a $[Ca^{2+}]_i$-releasing reagent with a protein synthesis or kinase inhibitor are routinely used to activate porcine and bovine eggs. The use of cyclohexamide, a protein synthesis inhibitor, is fundamentally based on its ability to stall mRNA translation on ribosomes, to prevent continuous cyclin B synthesis, required for maintenance of MPF activity and MII arrest[39,60].

Use of certain pharmacological agents also ensures inhibition of MPF activity, which includes CDK 1 selective inhibitors, roscovitine[55], olomoucine[70] and RO-3306[74] or the lower selectivity inhibitor, 6DMAP [67].

The latter that is 6DMAP has been used extensively and has been shown to decrease activity in bovine eggs and eggs of other species, including cattle[35], sheep[8], and pigs[88]. Although not proven, 6DAMP is thought to inhibit cdc25-phosphatase, which is responsible for activating CDK1 via dephosphorylation of Thr14 and Thr15 residues[17,75]. Moreover, this treatment resulted in higher rates of pre-implantation development of bovine SCNT embryos[40]. Unfortunately high rates of chromosomal abnormalities where observed in embryos generated by CHX and 6DMAP[90] treatment, the reason being broad and diverse specificities of these compounds[87,3,73]. Thus administration of these compounds requires careful attention to their detrimental effects on generated embryos.

Greater rates of chromosomal aneuploidy resulted from exposure to 6DMAP, which is directly in contrast to CHX treatment[73]. The use of specific CDK 1 inhibitors appears to be more reliable, although additional work is needed to determine the rate of activation by these inhibitors, as well as the "developmental competence" of the resultant embryos.

The combined exposure to ionomycin (IO) and CHX is effective in activating eggs derived from pigs as the developmental competence of SCNT and parthnogenetic embryos was substantially improved compared with that achieved by ionomycin alone[82]. Additionally, in these species exposure to thimerosal and DTT resulted in egg activation and consequent embryonic development [85,70]. Thimerosal is a thiol-oxidizing agent, which is capable of initiating $[Ca^{2+}]_i$ oscillations by sensitizing IP_3R1 and also presumably helping in Ca^{2+} transport inside the cell and inhibiting its export[9,68,42,87] Nevertheless incessant exposure to thimerosal has detrimental effects such as spindle disaasembly and continuous elevated levels of basal Ca^{2+}.

In conclusion, care should be exercised regarding the duration of exposure to thimerosal to induce egg activation in pig oocytes and this should immediately be followed by exposure to DTT, which possesses the ability to reverse protein modifications caused by thimerosal[70,83].

Egg Actvation in Humans:

SCNT has been used recently in humans with the goal of generating autologous organs for patients requiring cell or tissue therapy. In line with other species, human SCNT requires in vitro egg activation procedures.

Interestingly a method of parthenogenetic activation of ova has not emerged despite trying almost all methods of egg activation. Many issues have incidentally delayed research and development in this field, which includes difficulty in obtaining adequate number of eggs; poor or back dated experimental design, that failed to monitor $[Ca^{2+}]_i$ responses induced by the activation procedures; and the source of eggs as most preliminary studies were done on aging and failed-fertilized eggs[78].

The methods of artificial egg activation are in line with that of other species including electrical pulse stimulation[73], Ca^{2+}-ionophores [79,46,73] and media supplementation with $SrCl_2$[73]. These studies were generally directed towards parthenogenetic development, and the intricacies involved due to these treatments centered on pronuclear formation and/or blastocyst formation[34,51,54,61]. However as, mentioned above, these initial studies were performed in unfertilized eggs, derived from failed in vitro fertilization procedures or in vitro maturation (IVM) procedures. In spite of these diversions, all of these studies consistently exhibited that human eggs are not easily activated by simple $[Ca^{2+}]_i$-rises such as those induced after treatment with Ca^{2+}-ionophores[46,58,59], ethanol[46], or $SrCl_2$[73]. Strikingly, DC electrical pulses, or addition of a protein synthesis inhibitor in the culture media, seemed more appropriate in activating eggs derived from humans.

The limited success of the single-agent activation of eggs has fueled further research of other alternative approaches, especially pertaining to those methods, which was instrumental in activating eggs of other domestic species, such as the aforementioned "cocktail treatment"[67]. In line with treatment with the then available data from other such species, egg activation and embryo development occurred in human eggs after the so-called cocktail treatment. Although it should be emphasized at this juncture, that most of these studies were performed on freshly collected oocytes as against those available from previous studies, which were performed on aged eggs[67,11,82,47].

Therefore, a future goal should be to examine the $[Ca^{2+}]_i$ ion responses elicited by in vitro activation protocols, so that inferences can be drawn regarding the pivotal role of $[Ca^{2+}]_i$ or whether the stimulation is sufficient and whether it is a necessary prerequisite for egg activation. Several approaches have been taken to recreate the mode of activation by sperm and these approaches are applicable for human eggs. The adoption of this common parthenogenetic method has been hindered both by technical difficulties in preparation and delivery of the active product(s) and the consequent ambiguity in realizing the benefits of such treatment on embryonic development pertaining to $[Ca^{2+}]_i$ oscillations as against single $[Ca^{2+}]_i$ rises. Studies have highlighted that when mouse eggs that are activated by exposure to CHX with or without $[Ca^{2+}]_i$, substantially low developmental rates resulted[60].

It should be appreciated however that by reversing the conditions these deficiencies can be corrected, when the eggs are exposed to single or multiple rises in $[Ca^{2+}]_i$- rises, casting serious ambiguity on the beneficial effects of $[Ca^{2+}]_i$ ions on development. Further studies emphasize that both restricted and excessive $[Ca^{2+}]_i$ –stimulation negatively impacts both pre and post-implantation development of egg as well as somewhat altered gene expression in eggs[73].

Therefore, although highly attractive from the standpoint of reproducing the natural procedure of activation of egg by means of sperm, this approach demands careful implementation as limited or excessive stimulation both negatively influence subsequent egg development. While many questions still remain regarding how sperm influences $[Ca^{2+}]_i$-oscillations in eggs, an understanding is currently developing that the role of male specific PLCζ as the putative "trigger" responsible for Ca^{2+} oscillation**s[64]**. Injection of species-specific of PLCζ cRNAs initiated fertilization-like $[Ca^{2+}]_i$-oscillations in mammalian species which include, humans[59], bovine[39,62], porcine[85], rat[25,72], and equine[20,66].

Human PLCζ, was first identified in the year 2002, where injections of cRNA resulted in long lasting $[Ca^{2+}]_i$, oscillations in failed-fertilization aging human eggs[28]. Injection of recombinant human PLCζ was tried out in the year 2012[88] and induced $[Ca^{2+}]_i$-oscillations in both mouse and human eggs[48] (Fig.4). This has proven to be a viable option of activating human eggs in the absence of pharmacological agents. Another important effect that emerges from this experimental finding is that $[Ca^{2+}]_i$-induced oscillations, cause synchronized cytoplasmic contractions[86]. This can be detected by advanced imaging technologies, providing a non-invasive method to assess the presence and frequency of oscillations in real time[63,69]. Further analysis of these cytoplasmic contractions, have been used to examine developmental competence of the aforementioned zygotes[63].

Inducing Ca^{2+} ion oscillations inside eggs has been correlated with spatial and temporal expression of gene expression/DNA methylation profiles, as observed in developing embryos. Activation of SCNT in bovine eggs by PLCζ cRNA influenced the development of bovine embryos up to the blastocyst stage. Subsequent analysis of gene expression in eight cell and the following blastocyst stage embryos exhibited a similar expression pattern for a number of genes between IVF and PLCζ cRNA-generated embryos[63].

Additionally, levels of trimethylated lysine 27 of histone H3 (a change that is associated with silencing of gene expression) were substantially higher in nuclear transfer blastocyst generated using cycloheximide and DMAP (6-dimthyl-aminopurine) as against the scenario in eggs that are activated using PLCζ cRNA injection or IVF procedures[79].

Therefore these studies suggests that $[Ca^{2+}]_i$ oscillations induced by PLCζ-cRNA/recombinant protein is a suitable method of egg activation and objectives should be focused around the development of procedures that will aid in the preparation and delivery of this protein inside mammalian eggs[79].
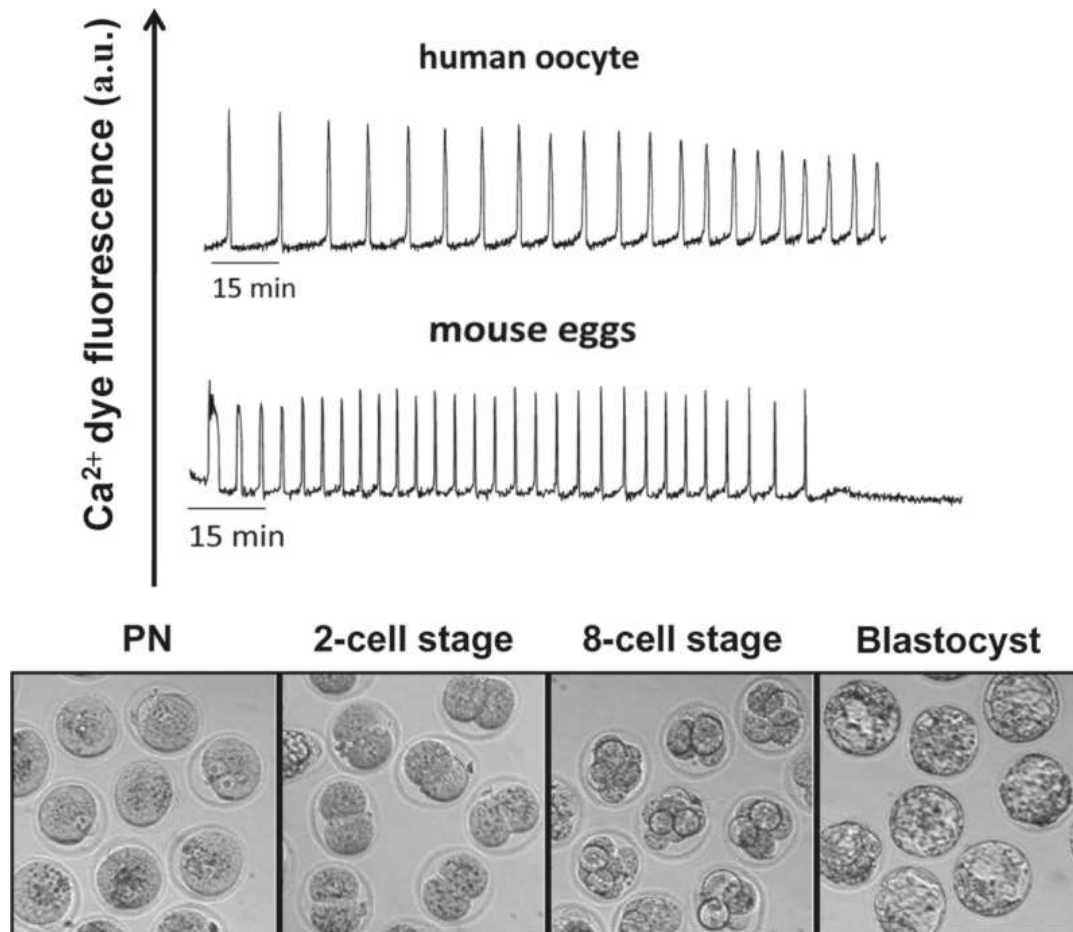

Fig.4: Early embryonic development in human and mouse eggs initiated by Ca^{2+} oscillations induced by the microinjection of recombinant human PLCζ protein. The upper panel shows representative fluorescence recordings illustrating the Ca^{2+} concentration changes in a human oocyte and a mouse egg following microinjection of human PLCζ recombinant protein. *Lower panel* shows micrographs illustrating mouse embryos at the various early developmental stages (pronuclear formation [PN], two-cell and eight-cell stages, and blastocyst stage) achieved after egg microinjection with ~80 fg of purified human PLCζ recombinant protein (Diagram courtesy: Nomikos M, Yu Y, Elgmati K et al, 2013).

3.   CONCLUSIONS AND FUTURE DIRECTIONS

The principal objectives of these aforementioned parthenogenetic methods are to induce the highest rate of activation of eggs, while at the same time, sponsoring pre and post-implantation development. Certainly application of $SrCl_2$ meets these criteria, as its mechanism of activation is dependent on oscillations that set in motion the "stepwise progression" of the developmental program, as is the case with sperm, and devoid of non-specific effects associated with treatment of eggs by broad-spectrum pharmacologic agents.

$SrCl_2$ is incapable of inducing egg activation in most mammals including humans. This finding not only inspires developmental biologists to unravel the putative mechanism(s) that underlie $SrCl_2$ influx to stimulate the development of improved methods of egg activation that do not demand supplementation with pharmacological agents.

The obstacle in the way of its adoption as a universal method of activation is the difficulty in delivery, as injection of eggs is rather cumbersome and the consequent damage that is inflicted on eggs may be substantial, dependent on the skill of the manipulator. The availability of PTDs or protein transduction domains and TAT, which are small peptides that are capable of penetrating biological membranes[23,84,89,69,67,71,80], that carry protein cargo in recent times, should be explored further so that the delivery of the most physiological stimulus for egg activation- PLCζ protein could be accomplished relatively easily not compromising with egg viability.

Moreover, recombinant human PLCζ protein could potentially be used in regenerative medicine approaches via generation of parthenogenetic embryos and blastocysts that may facilitate stem cell derivation and differentiation[24,49].

Considering the pivotal role of PLCζ to successful fertilization and consequent embryogenesis, it may represent a very significant biomarker to test the functioning of competent sperms. A recent investigation revealed after applying an improved technique viz., Motile Sperm Organelle Morphology Evaluation (MSOME), relying on high power magnification analysis of human sperm prior to application of an improved version of ICSI, namely IMSI. This may enable selection of sperm with higher concentration of PLCζ, as well as selecting a much higher proportion of sperms showing the presence of PLCζ[30].

Thus a significant application of PLCζ may be its use as a potent and important prognostic marker of sperm-oocyte activation capability, thus "FERTILITY". PLCζ analysis may also identify cases of male "sub-fertility", as compared to analysis from routine clinical semen parameters, pinpointing to the fact that analysis of PLCζ status may in the long run benefit the wider male population and not just cases of ICSI-failure[30].

Ionophore treatment may not be the viable option to overcome failure of egg activation. The reason for this is it may pose serious concerns for the overall health of the future embryo due to cytotoxic, mutagenic and teratognioc effects on the eggs and embryos[90,45].

The recent development in nanosciences and the possible application of nanoparticles for the introduction of PLCζ inside may be a viable option in the future. Nanoparticles, as we know are extremely small and minute sized that can easily cross the lipid barrier of most cells can be the ideal delivery system of PLCζ for egg activation.

ACKNOWLEDGEMENTS

Conflicts of Interests:

The authors do not have any direct financial relation that may lead to a conflict of interests for any of the authors mentioned herein, and there is no conflict of interests regarding the publication of this article.

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