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2 **Fertilization 2: Polyspermic fertilization**3 **Shusei Mizushima**4 **E-mail: smizus@sci.hokudai.ac.jp**5 **Department of Biological Sciences, Faculty of Science, Hokkaido University, Kita 10**6 **Nishi 8, Kitaku, Sapporo, Hokkaido 060-0810**

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Abstract

During fertilization in animals, a haploid egg nucleus fuses with a haploid sperm nucleus to restore the diploid genome. In most animals including mammals, echinoderms, and teleostei, the penetration of only one sperm into an egg is ensured at fertilization because the entry of two or more sperm is prevented by polyspermy block systems in these eggs. On the other hand, several animals such as birds, reptiles, and most urodele amphibians exhibit physiological polyspermy, in which the entry of several sperm into one egg is permitted. However, in these polyspermic eggs, only one sperm nucleus is involved in zygotic formation with a female nucleus, thereby avoiding syngamy with multiple sperm nuclei. In the chicken, 20-60 sperm are generally found within the egg cytoplasm at fertilization and this number is markedly higher than that of other polyspermic species; however, avian-specific events such as the degeneration and mitosis of supernumerary sperm nuclei during early embryo development allow a polyspermic egg to develop normally. This chapter describes current knowledge on polyspermy-related events in avian eggs during fertilization, and is characterized by a comparison to the fertilization modes of other vertebrates. The close relationship between sperm numbers and egg sizes, and the movement of supernumerary sperm nuclei towards the periphery of the egg cytoplasm and their degeneration are summarized. The molecular mechanisms by which polyspermy initiates egg activation to start embryo development are also discussed.

Key words: Diploid genome, Polyspermy block, Physiological polyspermy, Supernumerary sperm, Fertilization modes, Sperm numbers, Egg sizes, Egg activation

7.1

Polyspermy and embryo development

Fertilization is an indispensable event in the restoration of diploid genomes and the initiation of several reactions that lead to embryonic development in all animals. Most animals exhibit monospermy, in which a single sperm has the ability to activate the reaction important for early development (Swann 1996; Stricker 1999; Runft et al. 2002; Yanagimachi 2005).

Conversely, the simultaneous fertilization of an egg by two or more sperm is a lethal condition leading to aneuploidy and developmental arrest. Polyspermy in humans mostly results in spontaneous abortion. Although the birth of triploid or tetraploid children has been

reported, severe malformations and multiple abnormalities are associated with polyploidy births (Uchida and Freeman 1985; Sherard et al. 1986; Shiono et al. 1988; Roberts et al. 1996; Dean et al. 1997; Sun 2003). On the other hand, several sperm have been observed in porcine eggs at a high incidence under physiological conditions or *in vitro* fertilization (Sun and Nagai 2003); however, porcine egg cytoplasm has the ability to remove successive sperm (Xia et al. 2001), such that some polyspermic pig eggs may develop to term if successive sperm do not interrupt the diploid zygotic genome (Han et al. 1999). Polyspermic fertilization in this species is an extraordinary case, and, in most cases of monospermic fertilization in mammals, defenses against polyspermy are rapidly established after penetration of the first sperm (Galeati et al. 1991; Yanagimachi 1994).

The number of sperm reaching the egg surface is reduced during passage through the female reproductive tract and the egg extracellular matrix, zona pellucida, jelly layer, and vitelline envelope. In mice, only 100 or 200 out of the 50 million sperm ejaculated were previously shown to reach the ovulated egg (Wassarman 1994). Although the possibility of the penetration of multiple sperm into an egg decreases at fertilization, eggs still remain at risk of polyspermy. Therefore, most eggs have established a membrane block and zona pellucida block in order to prevent polyspermy (Yanagimachi 1994; Abbott and Ducibella 2001). Following sperm penetration, the rapid and transient depolarization of membrane potential at the level of the plasma membrane (membrane block) is elicited in order to prevent the fusion of additional sperm in the frog, *Xenopus laevis*, and several marine invertebrates (Jaffe and Gould 1985; Gould and Stephano 2003). This change in membrane potential is not observed in fertilized mouse, hamster, rabbit, or bony fish eggs, in which the membrane block employs a different mechanism to changes in membrane potential (Nuccitelli 1980; Miyazaki and Igusa 1981; Igusa et al. 1983; Jaffe et al. 1983; McCulloh et al. 1983). After a temporal fast block, the contents (e.g. ovastacin in human and mouse; Quesada et al. 2004; Liu 2011; Burkart et al. 2012) of cortical granules, a special organelle in eggs, are released into the perivitelline space via their exocytosis (a cortical reaction). Although the structure and molecules involved in the zona reaction differ among species, this reaction makes the zona pellucida refractory to the binding and fusion of a second sperm (the zona reaction), which is common to all vertebrate species (Wong and Wessel 2006). An egg must recognize the binding and fusion of the first fertilizing sperm and rapidly establish a polyspermy block. Therefore, the fertilizing sperm must immediately activate and propagate a signal cascade throughout the whole egg. Although the molecular mechanisms underlying the activation of a

1 polyspermy block have not yet been elucidated in detail, fertilization-mediated Ca^{2+} release from
2 intracellular stores and the activation of protein kinase C (PKC) are known to be involved in the
3 establishment of two different polyspermy blocks (Sun 2003; Gardner et al. 2007).

4 The eggs of some species, including ctenophores, elasmobranchs, urodele amphibians, reptiles,
5 and birds, physiologically permit the penetration of more than one sperm into the ooplasm at
6 fertilization (Elinson 1986; Iwao 2000; Wong and Wessel 2006; Snock et al. 2011; Mizushima
7 2012). Unlike monospermic eggs, neither a membrane block nor intracellular organelles similar
8 to cortical granules have been observed in these polyspermic eggs; therefore, there is no block
9 against the entry of a second or more sperm after the first sperm is incorporated into the egg
10 cytoplasm. Since only one sperm is eventually involved in zygotic formation with a female
11 pronucleus, embryo development with a diploid configuration is ensured. During natural
12 fertilization, the avian egg receives a markedly higher number of sperm than other
13 polyspermic species (Fig.8.1). Although it is technically difficult to calculate the total number
14 of sperm in the germinal disc, it appears likely that, at the lowest estimate, 20 sperm are
15 typically incorporated into an egg (Harper 1904; Patterson 1910, Fofanova 1965; Nakanishi et
16 al. 1990; Waddington et al. 1998). The maximum number of sperm found within the germinal
17 disc was previously reported to be 25 for the pigeon and 62 for the chicken (Harper 1904;
18 Nakanishi et al. 1990). Furthermore, more than 70 sperm were detected in the quail egg
19 within 1 hour of fertilization (our unpublished data).

20 The reason why the avian ovum may accept the entry of numerous sperm remained unclear
21 until recently. The findings of recent experiments on birds suggest that the number of sperm
22 incorporated into an egg cytoplasm affects the fate of the egg. The rate of chicken and turkey
23 embryos developing to the blastoderm stage was found to be approximately 50% when
24 approximately 3 sperm penetrated the inner perivitelline layer over the germinal disc region
25 (in this case, the number of sperm that penetrated the egg cytoplasm was estimated to be less
26 than 3), while the probability of egg development was almost 100% when more than 6 sperm
27 penetrated the region (Bramwell et al. 1995; Wishrt 1997). This finding suggests that the
28 fertilization rate increases when the number of sperm incorporated into an egg is higher. In
29 addition, the direct injection of sperm into the cytoplasm of a mature quail egg, namely, an
30 intracytoplasmic sperm injection (ICSI), directly revealed that a single sperm was insufficient
31 for a high rate of fertilization and subsequent blastoderm development (approximately 20%;

Hrabia et al. 2003; Takagi et al. 2007a; Mizushima 2008, 2009, 2012; Shimada et al. 2014; Kang et al. 2015). It has been proposed that the purpose of polyspermy is to increase the opportunity of a sperm nucleus migrating to the center of the germinal disc and making contact with the female nucleus because the surface area of the avian germinal disc in relation to that of sperm is very large. However, the ICSI technique revealed that the microinjection of a single sperm together with soluble protein equivalent to multiple sperm induced fertilization and subsequent development up to the blastoderm stage in 70% of eggs tested (Mizushima et al. 2014). Therefore, instead of the frequency of migrating sperm nuclei, an alternative hypothesis in which many spermatozoa are necessary to provide sufficient amounts of sperm-derived egg-activating proteins to ensure the successful initiation of egg development in birds is currently considered the most likely theory (see Sect. 8.2 for more details). On the other hand, there may be a physiological limitation to excessive polyspermy in the avian egg. For example, an increase in the number of sperm that make contact with the ovum by intramaginal insemination, which is greater than the normal numbers of sperm, induces an increase in the frequency of early blastoderm developmental arrest (Van Krey et al. 1966). More critically, a recent study showed that cytoplasmic segmentation occurs at a high rate in the absence of nuclear divisions in the germinal discs of quail and chicken ova inseminated *in vitro* using $1-2 \times 10^4$ semen, and eggs underwent development to the early pseudo-blastoderm stage (Olszanska et al. 2002; Batellier et al. 2003), indicating that an excessive number of supernumerary sperm interferes with normal fertilization and subsequent embryo development (Fechheimer 1981; Olszanska et al. 2002; Mizushima et al. 2009). A novel structure coated with calcium carbonate in the infundibulum part of the female reproductive tract in which fertilization occurs and sperm are stored until fertilization, termed the sperm-associated body (SB), was identified in domestic birds, and SB-accompanied sperm only were found to pass through the vitelline membrane (Sultana et al. 2004; Rabbani et al. 2006, 2007). This SB may contribute physiologically to reducing the number of excessive fertilizing sperm.

7.2

Egg activation

7.2.1

Ca²⁺ increase during fertilization

Physiological polyspermy occurs in oviparous species that exhibit internal fertilization such as cartilaginous fishes, urodele amphibians, reptiles, and birds (Elinson 1986; Iwao 2000;

Wong and Wessel 2006; Snook et al. 2011). Fertilizing sperm provide a signal to trigger the initiation of egg development (egg activation) as well as the nucleus because the development of animal eggs is arrested at the species-specific phase of meiosis until fertilization, e.g. at metaphase of second meiosis (metaphase II) in most vertebrate eggs (Stricker 1999). In all nomospermic and polyspermic animals studied to date, an increase in intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) has been observed immediately after sperm-egg binding or fusion during fertilization (Stricker 1999; Runft et al. 2002), and this increase in $[\text{Ca}^{2+}]_i$ plays a pivotal role in restarting cell cycle events in the egg, which comprise the resumption of meiosis and extrusion of the second polar body (Ducibella et al. 2002; Miyazaki 2006). Furthermore, in mammalian species, the establishment of polyspermy prevention is also evoked by an increase in $[\text{Ca}^{2+}]_i$ (McAvey et al. 2002; Gardner et al. 2007). In spite of its universality, the spatiotemporal patterns of the Ca^{2+} signal associated with egg activation vary widely among species (Stricker 1999). In monospermic fishes and the frog, *Xenopus laevis*, a transient increase in $[\text{Ca}^{2+}]_i$ induced by a single sperm was shown to propagate throughout the whole egg from the sperm-entering position as a Ca^{2+} wave (Ridgway et al. 1977; Busa and Nuccitelli 1985; Fluck et al. 1991; Abraham et al. 1993; Keating et al 1994; Creton et al. 1998). An increase in $[\text{Ca}^{2+}]_i$ during the activation of mammalian eggs is known to occur periodically in the form of long-lasting oscillations, which are known as Ca^{2+} oscillations (Miyazaki et al. 1993; Jones et al. 1995), and continues to approximately the first interphase stage, similar to that in mouse eggs (Jones et al. 1995; Marangos et al. 2003), or to the first mitotic cell cycle, as observed in bovine eggs (Nakada et al. 1995).

In mice, artificial stimuli such as electrical pulses or exposure to ethanol have revealed that a single Ca^{2+} pulse induces second polar body extrusion, but causes only partial egg activation due to the incomplete inactivation of cytosolic factor, which is the cytosolic protein responsible for meiotic arrest at metaphase II (Tatone et al. 1999; Ducibella et al. 2002; Jones 2005). In contrast, continuous stimuli for 24 hours fully activate eggs, which include an increase in cortical granule exocytosis and the formation of both polar bodies (Deguchi and Osanai 1995; Lawrence et al. 1998; Ozil 1998; Ducibella et al. 2002; Jones 2005, 2007). Accordingly, the mammalian egg needs to be exposed to a series of repetitive Ca^{2+} pulses in order to ensure it escapes meiosis because a number of hours are needed for a lapse between meiotic resumption and the interphase stage. Alternatively, unlike mammals, fertilization in fishes and *Xenopus laevis* triggers a rapid transition to pronuclear formation (Rugh 1951; Iwamatsu and Ohta 1978). The short

post-fertilization phase in these zygotes may obviate the second or more Ca^{2+} oscillations (Jones 1998).

In physiological polyspermic eggs, a few sperm successively enter at different points on the egg surface, and increases in $[\text{Ca}^{2+}]_i$ spread concentrically into the egg cytoplasm at each sperm entry site as small waves (Harada et al. 2011; Iwao 2012). However, unlike monospermic eggs such as those of fishes, *Xenopus laevis*, and mammals, each Ca^{2+} wave does not reach the opposite site of the egg. The $[\text{Ca}^{2+}]_i$ intensity in the entire egg region of polyspermic eggs, as revealed by a Ca^{2+} -sensitive fluorescence dye, shows a slow increase that continues for approximately 40 min, with the peak level reached being markedly lower than in *Xenopus laevis* eggs (Fontanilla and Nuccitelli 1998). In addition, a slow wave-like increase in $[\text{Ca}^{2+}]_i$ is also caused by a single injection of sperm extract (SE) containing soluble proteins into the newt egg cytoplasm, which propagates concentrically from the injection site through the entire egg without a local small wave, but is also able to evoke meiotic resumption (Harada et al. 2007). In polyspermy of the frog, *Discoglossus*, an increase in $[\text{Ca}^{2+}]_i$ lasts for 50 min after fertilization (Nuccitelli et al. 1988). However, several rapid spike-like depolarizations caused by sperm entry precede the major depolarization mediated by Ca^{2+} -activated Cl^- efflux (Talevi 1989), suggesting that a non-propagative small Ca^{2+} increase occurs at each sperm entry point in advance of the major Ca^{2+} wave (Iwao 2012).

In birds, a microinjection of SE into an ovulated egg retrieved from the infundibulum or upper part of the magnum revealed a very unique pattern for the increase in $[\text{Ca}^{2+}]_i$ in the quail egg, which has not been observed in other species (Fig.8.2). Its Ca^{2+} signal pattern is classified as two different kinds of Ca^{2+} waves, namely, a transient, slow wave and multiple, spiral-like oscillations (Mizushima et al. 2014). The slow Ca^{2+} wave was observed immediately after the microinjection, and an increase in $[\text{Ca}^{2+}]_i$ was initiated at the injection site of the germinal disc and its Ca^{2+} wave spread concentrically into the egg cytoplasm, but was restricted to the germinal disc. The increase in $[\text{Ca}^{2+}]_i$ at the injection site continued for approximately 5 min, and then decreased gradually before returning to the basal level within 30 min of the microinjection. On the other hand, an initial spiral-like Ca^{2+} signal occurred at the injection site 10-15 min after the microinjection, which was prior to the restoration of increases in $[\text{Ca}^{2+}]_i$ by the Ca^{2+} wave to the basal level. Second and further spiral-like Ca^{2+} waves originated successively before the disappearance of the previous Ca^{2+} spiral with a mean interspike interval of less than 1 min, and its oscillation lasted for at least 3 hours. Furthermore,

a few Ca^{2+} spirals overlapping partially in one egg were found to have complicated waveforms, which markedly differed from the Ca^{2+} oscillations observed during mammalian egg activation. It is important to note that these two different $[\text{Ca}^{2+}]_i$ patterns play different roles in the activation of the quail egg. A slow Ca^{2+} wave has the ability to evoke the resumption of meiosis and subsequent zygote formation (Mizushima et al. 2007, 2008, 2014), whereas spiral-like Ca^{2+} oscillations are not involved in these events. However, the induction of spiral-like Ca^{2+} oscillations in a slow Ca^{2+} wave-generated ICSI quail egg showing developmental arrest at the early embryo stage (Mizushima et al. 2008, 2010, 2014) activate full-term development to hatching, suggesting that spiral-like Ca^{2+} oscillations are necessary for cell cycle progression in the advanced zygote stage. More than 20 sperm generally enter chicken and quail eggs and then progressively form male pronuclei between 1 and 3 hours (Perry 1987; Nakanishi et al. 1990). Although the spatiotemporal changes that occur in $[\text{Ca}^{2+}]_i$ in the avian egg following natural fertilization or *in vitro* insemination still remain unknown, multiple Ca^{2+} spirals appear to be necessary, in part, for a continuous stimulation to complete pronuclei formation besides the cell cycle stimulation (Fig. 8.4).

7.2.2

Egg size and sperm number

The microinjection of a whole sperm into a mammalian egg (ICSI), which avoids any membrane contact between sperm and the egg, generates a Ca^{2+} oscillation similar to that observed during fertilization, and this Ca^{2+} oscillation is also triggered by a microinjection of SE corresponding to the content of a single sperm (Homa and Swann 1994; Wu et al. 1997, 1998; Dong et al. 2000; Parrington et al. 2000; Tang et al. 2000). Thus, the quantity of egg-activating proteins contained in a single sperm is necessary and sufficient for fully activating the eggs of monospermic species. It is important to note that avian SE equivalent to approximately 1 sperm or a whole avian sperm possess the ability to initiate Ca^{2+} oscillations and subsequent zygotic formation in a mouse egg (Dong et al. 2000; Takagi et al. 2007b), but have a negligible effect on the activation of an avian egg (Hrabia et al. 2003; Takagi et al. 2007a; Mizushima et al. 2009, 2014). These findings led to the hypothesis that a large number of sperm may be necessary in order to provide egg-activating factors not fulfilled by a single sperm due to the large size of an avian egg in comparison to that of a mammalian egg. Indeed, the volume of quail egg cytoplasm is more than 1-2 μL , whereas that of the mouse is approximately 200 pL.

Egg-activating proteins equivalent to 100-200 sperm are required for fertilization and full-term development to hatching in quails, which supports the strong correlation between egg size and polyspermy (Birkhead et al. 1994; Mizushima 2012, 2014; Shimada et al. 2014; Kang et al. 2015).

Unlike mammals in which a sperm-triggered Ca^{2+} wave propagates through the entire mammalian egg, the Ca^{2+} wave generated by a single sperm only propagates in one-eighth to one-quarter of the egg surface in physiologically polyspermic newts (Harada et al. 2011, Iwao 2012). Consistent with the egg size theory, several sperm must enter from different points in order to increase $[\text{Ca}^{2+}]_i$ throughout the large eggs of newts. Similarly, in polyspermy in birds, the propagation of a Ca^{2+} signal by a single sperm may not reach the whole egg, and, as such, a larger number of sperm than that needed by newts is required for the activation of the whole egg because the size of an avian egg is markedly larger than those of newts (Fig.8.4).

Although little is known about the discrepancy that a larger amount of SE (equivalent to 100-200 sperm) than that from the 2 to 60+ observed during natural fertilization is required for the full-term development of quail eggs following ICSI with a single sperm (Mizushima et al. 2014), these differences lie in the fact that 2-20 sperm typically penetrate newt eggs *in situ* (Iwao et al. 1985, 1993), whereas complete egg activation by a microinjection of newt SE requires a protein content equivalent to 330 sperm (Harada et al. 2011). Regarding egg activation, although limited information is available on the minimum number of sperm needed for the full activation of an avian egg, at least 20 sperm may be required to increase $[\text{Ca}^{2+}]_i$ throughout the entire avian egg. Otherwise, egg size may influence where and when sperm enters, and, thus, potentially affect the timing of sperm aster formation and migration of potential microtubule organization centers (MTOCs), which are responsible for the movement of the pronucleus to karyogamy. The extra space in the egg cytoplasm of large eggs is one of the most important factors for eliminating supernumerary sperm nuclei in the same egg cytoplasm (Elinson 1986; Iwao 2012). Based on various aspects of internal fertilization, centrosome dynamics, evolutionary history, and egg cytological polyspermy blocks (see Sect. 8.3 for details), the close relationship between increased egg sizes and the number of sperm in the acquisition of polyspermy needs to be discussed in more detail (Snook et al. 2011). This evidence based on an

7.2.3

Molecular mechanisms underlying egg activation in physiological polyspermy

1 An increase in $[Ca^{2+}]_i$ in the eggs of vertebrates is initiated by Ca^{2+} release via the inositol
 2 1,4,5-trisphosphate (IP_3) receptor (IP_3R). A large number of studies on vertebrate eggs have
 3 demonstrated that an injection of IP_3 induces the release of Ca^{2+} from Ca^{2+} stores, mainly the
 4 endoplasmic reticulum, in the egg (Miyazaki 1988; Miyazaki et al. 1992; Fissore and Robl
 5 1993; Swann and Ozil 1994; Wang et al. 1999; Amano et al. 2004; Lee et al. 2010;
 6 Mizushima et al. 2014). The repetitive increases observed in $[Ca^{2+}]_i$ (Ca^{2+} oscillations) during
 7 mammalian fertilization are no exception. IP_3 -induced Ca^{2+} release as well as Ca^{2+} -induced
 8 Ca^{2+} release, which operates by the sensitizing effects of Ca^{2+} on the IP_3R , contribute to the
 9 regenerative process of Ca^{2+} release (Miyazaki et al. 1993). Since IP_3 is generated by enzymes
 10 of the phospholipase C (PLC) family, which catalyze the hydrolysis of phosphatidylinositol
 11 4,5-bisphosphate (PIP_2) into IP_3 and diacylglycerol (Rhee 2001), two main molecular
 12 signaling models have been proposed for the production of IP_3 in the egg. The first suggestion
 13 proposes that sperm-egg fusion induces the activation of a receptor on the egg plasma membrane
 14 and couples to either a G-protein or tyrosine kinase (Src kinase), and this is followed by the
 15 activation of $PLC\beta$ or $PLC\gamma$, respectively (Runft et al. 2002). In *Xenopus laevis*, Src kinase is
 16 phosphorylated and then stimulates $PLC\gamma$ at the time of sperm-egg contact (Sato et al. 1999, 2000,
 17 2001, 2003). Sperm induces the transient phosphorylation of Uroplakin III (UPIII) on the egg
 18 membrane, which is a substrate of the egg cytoplasmic Src kinase, and Src (Sakakibara et al. 2005;
 19 Mahbub Hasan et al. 2014). In addition, a sperm-derived protease associated with a sperm surface
 20 glycoprotein (Nagai et al. 2009) serves as a target of the extracellular domain of UPIII (Mahbub
 21 Hasan et al. 2005; Sakakibara et al. 2005). Although the molecular signaling pathway for egg
 22 activation in the monospermy of the primitive jawless fish lamprey remains unknown, the
 23 involvement of a receptor on the egg membrane, and not sperm-egg fusion has been postulated
 24 because most eggs clamped at a positive potential (+20 to +40 mV) were found to undergo
 25 egg activation (Kobayashi et al. 1994).

26 An alternative hypothesis has been proposed for increases in $[Ca^{2+}]_i$ in which the sperm itself
 27 contains soluble egg-activating factors that diffuse directly into the cytoplasm of the egg after
 28 fusion. Since fertilization-induced increases in $[Ca^{2+}]_i$ are not prevented in the mouse egg in
 29 spite of the introduction of a G-protein-specific inhibitor or $PLC\gamma$ (Moore et al. 1993;
 30 Mehlmann et al. 1998; Williams et al. 1998), this appears to be the case in most mammalian
 31 species (Swann 1996; Stricker 1999; Runft et al. 2002). The most likely candidate for the
 32 initiator of an increase in $[Ca^{2+}]_i$ in mammalian eggs is a sperm-specific member of the PLC

1 isozyme, PLCzeta (PLC ζ ; Saunders et al. 2002). Although other sperm-inducing factors have
 2 been reported (Sette et al. 2002; Perry et al. 1999, 2000), they have not yet been substantiated
 3 and important evidence now exists to show that PLC ζ alone has the ability to generate Ca²⁺
 4 oscillations in mouse eggs at an amount equivalent to the content in a single sperm (Saunders
 5 et al. 2002; Kouchi et al. 2004). Furthermore, a PLC ζ orthologue has also been identified in
 6 humans, monkeys, rats, pigs, bovines, and horses as a common egg-activating factor in
 7 mammals (Cox 2002; Yoneda et al. 2006; Ito et al. 2008; Ross et al. 2008; Sato et al. 2013).
 8 The mouse PLC ζ contains a nuclear localization signal that promotes its accumulation in the
 9 pronuclei (Larman et al. 2004; Yoda et al. 2004; Kuroda et al. 2006). The nuclear
 10 accumulation of PLC ζ appears to terminate long-lasting Ca²⁺ oscillations. In addition, the
 11 sperm of the teleost fish, tilapia, contains an egg-activating factor for increasing Ca²⁺ in
 12 mouse eggs or sea urchin egg homogenates (Coward et al. 2003), and the medaka testes also
 13 contain PLC ζ that initiates Ca²⁺ oscillations in mouse eggs (Coward et al. 2011). However,
 14 PLC ζ in the pufferfish, Fugu, is expressed in the ovary and brain, but not in the testis, and its
 15 ovarian form does not have the ability to trigger Ca²⁺ oscillations in mouse eggs (Coward et al.
 16 2011). Therefore, PLC ζ does not appear to be involved in the sperm-induced activation of all
 17 fish eggs.

18 On the other hand, citrate synthase (CS) is the most likely candidate for an initiator of increases
 19 in [Ca²⁺]_i in newt eggs (Harada et al. 2007). Although the molecular mechanisms underlying
 20 the initiation of an increase in [Ca²⁺]_i by sperm CS have not yet been elucidated in detail, an
 21 experimental hypothesis was recently proposed in which CS released from sperm forms a
 22 complex with maternal microtubules and the ER with the IP₃R as well as PLC γ in the midpiece
 23 region of the sperm, and this complex acts on small Ca²⁺ wave propagation by the sequential
 24 activation of PLC γ in order to stimulate the IP₃R (Ueno et al. 2014). Or paternal CS cleaves citrate
 25 into acetyl-CoA and oxaloacetate in the egg cytoplasm, and the former and latter then trigger Ca²⁺
 26 release from the ER and mitochondria, respectively (Harada et al. 2011). In somatic cells, CS
 27 produces citrate from acetyl-CoA and oxaloacetate in the mitochondrial tricarboxylic acid (TCA)
 28 cycle, but may inversely cleave citrate, which is abundant in the egg cytoplasm, to produce
 29 acetyl-CoA and oxaloacetate (Srere 1992; Iwao, 2012). Increases induced in acetyl-CoA in the
 30 egg cytoplasm by sperm CS may sensitize IP₃Rs on the ER (Missiaen et al. 1997). Oxaloacetate
 31 has been suggested to have the potential to induce the release of Ca²⁺ from mitochondria (Leikin
 32 et al. 1993). Therefore, paternal CS functions as an enzyme in the egg in order to produce

acetyl-CoA and oxaloacetate and/or as a PLC γ and IP $_3$ R stimulator to generate the release of Ca $^{2+}$ from Ca $^{2+}$ stores. Furthermore, since SE derived from newts cannot generate Ca $^{2+}$ oscillations in the mouse egg, the mechanism responsible for egg activation in newts differs from PLC ζ signaling (Harada et al. 2007). A PLC ζ orthologue has not yet been identified in newts.

In contrast to PLC ζ and CS alone being sufficient to evoke the release of Ca $^{2+}$ in mammalian and newt eggs, at least 3 egg-activating factors, namely, PLC ζ , CS, and aconitate hydratase (AH), are essential for the complete release of Ca $^{2+}$ in order to activate the full-term development of avian eggs to hatching (Fig.8.3; Mizushima et al. 2014). Even though avian PLC ζ has the ability to generate Ca $^{2+}$ oscillations in the mouse egg (Coward et al. 2005), it is only involved in an initial slow Ca $^{2+}$ wave in quail eggs (Mizushima et al. 2014). This difference in responses to PLC ζ in these eggs may be associated with the desensitization of the IP $_3$ R channel to IP $_3$ and

IP $_3$ -induced Ca $^{2+}$ resulting from PLC ζ activity, and not to any reductions in enzyme activity.

The IP $_3$ Rs in mammalian eggs are progressively desensitized by ubiquitination and subsequent proteasome activity, which corresponds to the termination of Ca $^{2+}$ oscillations at the interphase stage (Zhu et al. 1999; Brind et al. 2000; Zhu and Wojcikiewicz 2000; Malcuit et al. 2005; Lee et al. 2010), suggesting that IP $_3$ binding to IP $_3$ Rs initiates the down-regulation of IP $_3$ Rs to terminate PLC ζ -induced egg activation immediately after fertilization in birds.

Although limited information is available for the down-regulation of maternal IP $_3$ Rs during avian fertilization, the long-lasting spiral-like Ca $^{2+}$ oscillation initiated 10-15 min after the PLC ζ -induced Ca $^{2+}$ wave slowly occurs irrespective of the absence of IP $_3$ Rs because the introduction of an antagonist of IP $_3$ R did not prevent Ca $^{2+}$ oscillations in the quail (Mizushima et al. 2014). On the other hand, CS and AH are both responsible for the generation of spiral-like Ca $^{2+}$ oscillations. This spiral-like Ca $^{2+}$ oscillation is not induced by CS or AH alone. Egg-activating activity in SE was found to be abolished by a treatment with each antibody, which supports these findings. Spiral-like Ca $^{2+}$ oscillations induced by CS and AH may be partly ascribed to the release of Ca $^{2+}$ from ryanodine receptors on the ER because the microinjection of an agonist of ryanodine receptors into quail eggs was found to initiate repetitive Ca $^{2+}$ spikes similar to CS- and AH-induced spike-like Ca $^{2+}$ oscillations (Fig.8.3; Mizushima et al. 2014). Previous studies reported that ryanodine receptors participated in the Ca $^{2+}$ waves observed in sea urchin eggs during egg activation (Galione et al. 1993; Lee et al. 1993; Miyazaki et al. 2006). The elucidation of variations in egg activation systems and particularly increases in Ca $^{2+}$ at fertilization in

vertebrates may contribute to a more detailed understanding of the evolutionary history of egg activation concomitant with the acquisition of polyspermy (Table 8.1).

7.2.4

Variations in and evolution of the sperm factor

The egg-activating factors responsible for increases in $[Ca^{2+}]_i$ in eggs are characterized as sperm-specific molecular triggers in all vertebrates studied. Although AH and CS are originally mitochondrial genes involved in the TCA cycle, the molecular weights of proteins detected in sperm are slightly higher for CS and lower for AH than those in body cells (Mizushima et al. 2014). Since their predicted amino acid sequences are partially different from those in somatic cells, their sperm-specific isoforms appear to be diversified and specified by gene duplication as an egg-activating factor. The molecular weight of sperm-specific newt CS was also previously shown to be slightly higher than that of other tissues (Harada et al. 2007), and this difference has been attributed to the hyper-phosphorylation of sperm CS (Ueno et al. 2014). Unlike PLC ζ in monospermic species, the acquisition of novel egg-activating factors in the polyspermic species, quails and newts, may have played a pivotal role in the evolution of slower activation in polyspermic eggs and may also have promoted the reproductive isolation necessary for speciation in vertebrates. However, a review of variations in sperm-specific CS and AH between quails and newts indicates divergent evolution in the molecular mechanisms underlying increases in $[Ca^{2+}]_i$ concomitant with a species-specific transition in the mode of polyspermic fertilization. A previous study showed that a microinjection of quail CS alone into a homogeneous egg did not have the ability to generate Ca^{2+} waves (Mizushima et al. 2014).

On the other hand, PLC ζ is solely expressed in the sperm of most animals such as mammals (Cox et al. 2002; Saunders et al. 2002; Yoneda et al. 2006; Young et al. 2009), birds (Coward et al. 2005; Mizushima et al. 2008, 2009), and medaka (Ito et al. 2008). This ensures the sperm-specific enrichment of egg-activating factors, consistent with a gamete-specific role. Genomic DNA analyses have revealed that the PLC ζ gene is located back to back with another testis-specific gene, the actin-capping protein gene *CAPZA3* (Hurst et al. 1998; Yoshimura et al. 1999; Miyagawa et al. 2002), which is inserted in the 5'-region of PLC ζ and shares a bidirectional promoter with PLC ζ (Coward et al. 2005, 2011), suggesting that the expression of CAPZA3 and PLC ζ is male germ cell- or testis-specifically transcribed in the same process. However, the PLC ζ of puffer fish was expressed in the brain and ovaries, but not in the testes. More interestingly, the

CAPZA3 genes of puffer fish and medaka were not adjacent to the PLC ζ genes (Coward et al. 2011). Although difficulties are associated with accounting for variations in the tissue-specific expression patterns of PLC ζ in these fishes, during the rapid evolution of egg activation mechanisms, PLC ζ seemed to be the first important differentiation factor for sperm function in the vertebrate species. Pufferfish PLC ζ does not have the ability to activate the release of Ca²⁺ in mouse eggs; however, this does not exclude the possibility that the activation of PLC ζ by a fertilizing sperm functions as an egg-derived Ca²⁺-releasing factor and/or plays some other role in the egg. Further investigations will provide an insight into the functional role and genesis of PLC ζ .

PLC ζ has not yet been detected in intermediate species between fishes and higher eutherian mammals, such as primitive mammals including the *monotrematous* platypus and small marsupial mammals (Table 8.1). The platypus, *Ornithorhynchus anatinus*, lays large yolky eggs that exhibit polyspermy, and several sperm have been suggested to enter the egg cytoplasm (Gatenby and hill 1924; Hughes and Hall 1998). Furthermore, the eggs of the small marsupial mammal, *Sminthopsis crassicaudata*, are relatively small, but occasionally exhibit polyspermy (Breed and Leigh 1990). Although changes in [Ca²⁺]_i at fertilization have not yet been examined in primitive mammals, egg activation in the ancestors of mammals may be achieved by polyspermy, with eggs showing primitive Ca²⁺ oscillations. Since the platypus, the most primitive mammal, shares common molecular, genetic, and morphological features with birds and reptiles (Rens et al. 2007) and are in a comparatively close cluster taxonomically (Warren et al. 2008), the spiral-like Ca²⁺ oscillations observed in avian egg activation may be closely related to an ancestor of mammalian Ca²⁺ oscillations. In this respect, these primitive mammalian species may possess a PLC ζ orthologue or intermediate molecules similar to three egg-activating factors.

7.3

Syngamy and elimination of supernumerary sperm in the egg cytoplasm

In monospermic species, frog, and *Hynobium* salamander eggs, increases in Ca²⁺ open Ca²⁺-dependent Cl⁻ channels on the egg plasma membrane in order to produce rapid depolarization, which prevents the penetration of additional fertilizing sperm (Cross and Elinson 1980; Iwao 1989; Iwao and Jaffe 1989). Previous studies demonstrated that rapid depolarization or a cortical reaction does not occur in polyspermic urodeles (Charbonneau et al. 1983; Iwao 2012). In polyspermic frog, *Discoglossus* eggs exhibit a fertilization-activated membrane

potential due to the opening of Cl^- channels; however, it does not block additional sperm entry (Talevi 1989). On the other hand, hyperpolarization mediated by Na^+ channels in response to each sperm penetration has been indicated in polyspermic newt eggs (Iwao 1985), but does not prevent second sperm penetration (Iwao and Jaffe 1989). Another polyspermy block system, the cortical reaction described above, is well-developed in mammalian eggs (Yanagimachi 1994; Quesada et al. 2004; Wong and Wessel 2006; Liu 2011; Burkart et al. 2012), whereas the dynamic movement and exocytosis of cortical granules are not likely in newt eggs (Iwao 2000). Although electrical responses in avian eggs have not yet been examined, a review of findings obtained using several amphibian eggs suggests that there is neither electrical regulation on the surface of egg plasma membranes nor alterations in the extracellular matrix that prevent polyspermy in all polyspermic species.

Even though numerous sperm are incorporated into polyspermic eggs, the egg nucleus proceeds to karyogamy with a single sperm nucleus because of the presence of an ooplasmic block to escape polyploidy. In birds, a large number of sperm penetrate the egg cytoplasm, most of which undergo transformation into male pronuclei after swelling, a change from the elongated form to the spherical head, chromatin decondensation, and reconstitution of the nuclear envelope (Fofanova 1965; Okamura and Nishiyama 1978; Perry 1987; Nakanishi et al. 1990; Waddington et al. 1998). However, supernumerary sperm (accessory sperm) nuclei, except for the one (principal sperm) that unites with the female pronucleus to form a zygote, move towards the periphery of the germinal disc, it is not in the yolk, but in the vicinity of cytoplasm, and supernumerary sperm nuclei undergo one or two mitoses (Perry 1987). This dynamic movement of accessory sperm in avian eggs has not been observed in other polyspermic species. The mitosis of accessory sperm occurs in synchrony with the zygotic nucleus, and the resultant cell division at the peripheral region of the egg cytoplasm disappears during the early cleavage stage (Paterson 1910; Eyal-Gilidi and Kochav 1976). Such nuclear division without DNA synthesis may represent a deviation from periodic nuclear activation (Emanuelsson 1965; Gurdon and Woodland 1968). It is important to note that, in addition to this, since ICSI with a single sperm, without accessory sperm-activating temporal cleavage, has been shown to develop quail eggs to the late blastoderm stage (Hrabia et al. 2003; Takagi et al. 2007; Mizushima et al. 2007, 2008, 2012, 2014; Kang et al. 2015), accessory sperm nuclei are not essential for any cellular events related to zygotic development. Although the molecular mechanisms suppressing supernumerary sperm nuclei have not been

1 elucidated fully, the involvement of maternal-derived deoxyribonucleases (DNase I and II) in
2 birds, which are not expressed in monospermic mammalian eggs (Stepinska et al. 2001, 2003;
3 Olszanska and Stepinska 2008), is under debate.

4 On the other hand, the mechanisms by which the movement of the male and female pronuclei
5 forms the zygote nucleus remain obscure in birds. This assures that only one sperm is selected
6 as the principal sperm by some unknown mechanisms and is subsequently paired with the
7 female pronucleus in the center of the germinal disc 3-4 hours after fertilization (Fig.8.4;
8 Perry 1987). Female metaphase II chromatin is localized in the superficial cytoplasm near the
9 center of the germinal disc when the avian egg is ovulated (Perry 1987); however, even in the
10 presence of numerous sperm, the female pronucleus still occupies a central position (Perry
11 1987), indicating that the selected principal sperm pronucleus appears to move toward the
12 female pronucleus. In fertilized newt eggs, only one sperm nucleus, possibly that nearest to the
13 female nucleus, forms a larger sperm aster than that of accessory sperm, and then makes contact
14 with the female pronucleus in the center of the animal hemisphere (Iwao et al. 2002). The maternal
15 γ -tubulin predominantly distributed in the animal hemisphere strongly accumulates in the
16 centrosomes of the one principal sperm nucleus and subsequent zygote nucleus in order to
17 promote microtubule polymerization, whereas only a small amount of γ -tubulin is associated
18 with those of other sperm nuclei (Iwao et al. 2002; Morito et al. 2005). γ -tubulin is a
19 well-known major component of the MTOC, and is involved in the movement of the male
20 pronucleus to fuse with the female pronucleus (Haren et al. 2006; Eot-Houllier et al. 2010),
21 suggesting that γ -tubulin is one of the key factors in the event related to the selection of the
22 principal sperm nucleus in newts (Reinsch and Karsenti 1997; Iwao et al. 2002). Furthermore,
23 since the principal sperm and female nuclei enter the DNA synthesis phase of the first cleavage
24 earlier than the accessory sperm nuclei, zygotic nuclei can enter the mid-term of the first cleavage.
25 This is because cyclin B that forms the MPF complex accumulates in and disappears from the
26 zygotes earlier than accessory sperm cells (Iwao and Elinson 1990; Iwao et al. 2002). The failed
27 progression of cyclic nuclear activities in accessory sperm may be due to insufficient exposure
28 of the MPF complex (Iwao et al. 1993; Sakamoto et al. 1998). In addition to the possible
29 participation of the motor proteins, dynein, dynactin, and kinesin in opposite movements
30 between the principal and accessory sperm nuclei (Payne et al. 2003; Waitzman and Rice 2014),
31 further investigations on the sequential distribution of avian cdc2 (Mori et al. 1991) and cyclin

1 B as well as γ -tubulin in the egg will provide insights into not only the selection of principal
2 sperm nuclei, but also the cellular process of zygotic fusion.

4 **7.4**

5 **Conclusion**

6 Development of avian ICSI technique has brought us a new schematic diagram in avian
7 fertilization (Fig.8.3). However, new finding such as novel sperm-derived egg-activating
8 factors is only a part of mysterious events of polypsermic fertilization. Therefore, more
9 information will be needed in order to understand the comprehensive molecular mechanism of
10 avian fertilization. Experiments using gene-disrupted animals are very powerful tools for
11 validating which factors are essential, which has also contributed to find novel genes in many
12 species. In particular, in vitro fertilization study is one of the suitable research fields to use
13 gene-manipulated animals. Fortunately, the TALEN and Crisper/Cas 9 systems have opened a
14 new door for avian gene-disruption (see Sect.13 for more details). The combination of avian
15 ICSI and gene-manipulation systems will make a significant progress in our understanding of
16 avian fertilization system.

References

- Abbott AL, Ducibella T (2001) Calcium and the control of mammalian cortical granule exocytosis. *Front Biosci* 6:D792–806. doi:10.2741/A643
- Abraham VC, Gupta S, Fluck RA (1993) Ooplasmic segregation in the medaka (*Oryzias latipes*) egg. *Biol Bull* 184(2):115–124.
- Amano T, Mori T, Watanabe T (2004) Activation and development of porcine oocytes matured in vitro following injection of inositol 1,4,5-trisphosphate. *Anim Reprod Sci* 80(1-2): 101-112. doi:10.1016/S0378-4320(03)00115-5
- Batellier F, Couty I, Olszanska B et al (2003) In vitro fertilization of chicken oocytes after in vitro ovulation. *Br Poult Sci* 44(5):819–820. doi:10.1080/00071660410001667014
- Birkhead TR, Sheldon BC, Fletcher F (1994) A comparative study of sperm-egg interactions in birds. *J Reprod Fertil* 101(2):353–361. doi:10.1530/jrf.0.1010353
- Bramwell RK, Marks HL, Howarth B (1995) Quantitative determination of spermatozoa penetration of the perivitelline layer of the hen's ovum as assessed on oviposited eggs. *Poult Sci* 74(11):1875–1883. doi:10.3382/ps.0741875
- Breed WG, Leigh CM (1990) Morphological changes in the oocyte and its surrounding vestments during in vivo fertilization in the dasyurid marsupial *Sminthopsis crassicaudata*. *J Morphol* 204(2):177–196. doi:10.1002/jmor.1052040207
- Brind S, Swann K, Carroll J (2000) Inositol 1,4,5-trisphosphate receptors are downregulated in mouse oocytes in response to sperm or adenophostin A but not to increases in intracellular Ca^{2+} or egg activation. *Dev Biol* 223(2):251–265. doi:10.1006/dbio.2000.9728
- Burkart AD, Xiong B, Baibakov B et al (2012) Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. *J Cell Biol* 197(1):37–44. doi:10.1083/jcb.201112094
- Busa WB, Nuccitelli R (1985) An elevated free cytosolic Ca^{2+} wave follows fertilization in eggs of the frog, *Xenopus laevis*. *J Cell Biol* 100(4):1325–1329. doi:10.1083/jcb.100.4.1325
- Charbonneau M, Moreau M, Picheral B et al (1983) Fertilization of amphibian eggs: a comparison of electrical responses between anurans and urodeles. *Dev Biol* 98(2):304–318. doi:10.1016/0012-1606(83)90361-5
- Coward K, Campos-Mendoza A, Larman M et al (2003) Teleost fish spermatozoa contain a cytosolic protein factor that induces calcium release in sea urchin egg homogenates and

- 1 triggers calcium oscillations when injected into mouse oocytes. *Biochem Biophys Res*
- 2 *Commun* 305(2):299–304. doi:10.1016/S0006-291X(03)00753-8
- 3 Coward K, Ponting CP, Chang HY et al (2005) Phospholipase C ζ , the trigger of egg
- 4 activation in mammals, is present in a non-mammalian species. *Reproduction* 130(2):157–163.
- 5 doi:10.1530/rep.1.00707
- 6 Coward K, Ponting CP, Zhang N et al (2011) Identification and functional analysis of an
- 7 ovarian form of the egg activation factor phospholipase C ζ (PLC ζ) in pufferfish. *Mol*
- 8 *Reprod Dev* 78(1):48-56. doi:10.1002/mrd.21262
- 9 Cox LJ, Larman MG, Saunders CM et al (2002) Sperm phospholipase C ζ from humans and
- 10 cynomolgus monkeys triggers Ca²⁺ oscillations, activation and development of mouse oocytes.
- 11 *Reproduction* 124(5):611–623. doi:10.1530/rep.0.1240611
- 12 Creton R, Speksnijder JE, Jaffe LF (1998) Patterns of free calcium in zebrafish embryos. *J*
- 13 *Cell Sci* 111(Pt 12):1613–1622.
- 14 Cross NL, Elinson RP (1980) A fast block to polyspermy in frogs mediated by changes in the
- 15 membrane potential. *Dev Biol* 75(1):187–198. doi:10.1016/0012-1606(80)90154-2
- 16 Dean J, Cohen G, Kemp J et al (1997) Karyotype 69, XXX/47, XX, +15 in a 2 1/2 year old
- 17 child. *J Med Genet* 34(3):246–249. doi:10.1136/jmg.34.3.246
- 18 Deguchi R, Osanai K (1995) Serotonin-induced meiosis reinitiation from the first prophase
- 19 and from the first metaphase in oocytes of the marine bivalve *Hiatella flaccida*: respective
- 20 changes in intracellular Ca²⁺ and pH. *Dev Biol* 171(2):483–496. doi:10.1006/dbio.1995.1298
- 21 Dong JB, Tang TS, Sun FZ (2000) *Xenopus* and chicken sperm contain a cytosolic soluble
- 22 protein factor which can trigger calcium oscillations in mouse eggs. *Biochem Biophys Res*
- 23 *Commun* 268(3):947–951. doi:10.1006/bbrc.2000.2218
- 24 Ducibella T, Huneau D, Angelichio E et al (2002). Egg-to-embryo transition is driven by
- 25 differential responses to Ca²⁺ oscillation number. *Dev Biol* 250(2):280–291.
- 26 doi:10.1006/dbio.2002.0788
- 27 Elinson RP (1986) Fertilization in amphibians: the ancestry of the block to polyspermy. *Int*
- 28 *Rev Cytol* 101:59–100. doi:10.1016/S0074-7696(08)60246-6
- 29 Emanuelsson H (1965) Cell manipulation in the chick blastoderm up to the time of laying.
- 30 *Exp Cell Res* 39(2):386–399. doi:10.1016/0014-4827(65)90042-X
- 31 Eot-Houllier G, Venoux M, Vidal-Eychenie S et al (2010) Plk1 regulates both ASAP
- 32 localization and its role in spindle pole integrity. *J Biol Chem* 285(38):29556–29568.

- 1 doi:10.1074/jbc.M110.144220
- 2 Eyal-Giladi H, Kochav S (1976) From cleavage to primitive streak formation: A
3 complementary normal table and a new look at the first stages of the development of the chick.
4 Dev Biol 49(2):321–337. doi:10.1016/0012-1606(76)90178-0
- 5 Fechheimer NS (1981) Origins of heteroploidy in chicken embryos. Poult Sci 60(7):1365–1371.
6 doi:10.3382/ps.0601365
- 7 Fissore RA, Robl JM (1993) Sperm, inositol trisphosphate, and thimerosal-induced
8 intracellular Ca^{2+} elevations in rabbit eggs. Dev Biol 159(1):122–130.
9 doi:10.1006/dbio.1993.1226
- 10 Fluck RA, Miller AL, Jaffe LF (1991) Slow calcium waves accompany cytokinesis in medaka
11 fish eggs. J Cell Biol 115(5):1259–1265. doi:10.1083/jcb.115.5.1259
- 12 Fofanova KA (1965) Morphologic data on polyspermy in chickens. Fed Proc Transl Suppl
13 24:239–247
- 14 Fontanilla RA, Nuccitelli R (1998) Characterization of the sperm-induced calcium wave in
15 *Xenopus* eggs using confocal microscopy. Biophys J 75(4):2079–2087.
16 doi:10.1016/S0006-3495(98)77650-7
- 17 Galeati G, Modina S, Lauria A et al (1991) Follicle somatic cells influence pig oocyte
18 penetrability and cortical granule distribution. Mol Reprod Dev 29(1):40–46.
19 doi:10.1002/mrd.1080290107
- 20 Galione A, McDougall A, Busa W et al (1993) Redundant mechanisms of calcium-induced
21 calcium release underlying calcium waves during fertilization of sea urchin eggs. Science
22 261(5119):348–352. doi:10.1126/science.8392748
- 23 Gardner AJ, Williams CJ, Evans JP (2007) Establishment of the mammalian membrane block
24 to polyspermy: evidence for calcium-dependent and -independent regulation. Reproduction
25 133(2):383–393. doi:10.1530/REP-06-0304
- 26 Gatenby JB, Hill JP (1924) On an ovum of *Ornithorhynchus* exhibiting polar bodies and
27 polyspermy. J Cell Sci s2–68(270):229–238
- 28 Gould MC, Stephano JL (2003) Polyspermy prevention in marine invertebrates. Microsc Res
29 Tech 61(4):379–388. doi:10.1002/jemt.10351
- 30 Gurdon JB, Woodland HR (1968) The cytoplasmic control of nuclear activity in animal
31 development. Biol Rev Camb Philos Soc 43(2):233–267.
32 doi:10.1111/j.1469-185X.1968.tb00960.x

- 1 Han YM, Wang WH, Abeydeera LR et al (1999) Pronuclear location before the first cell
2 division determines ploidy of polyspermic pig embryos. *Biol Reprod* 61(5):1340–1346.
3 doi:10.1095/biolreprod61.5.1340
- 4 Harada Y, Kawazoe M, Eto Y et al (2011) The Ca^{2+} increase by the sperm factor in
5 physiologically polyspermic newt fertilization: its signaling mechanism in egg cytoplasm and
6 the species-specificity. *Dev Biol* 351(2):266–276. doi:10.1016/j.ydbio.2011.01.003
- 7 Harada Y, Matsumoto T, Hirahara S et al (2007) Characterization of a sperm factor for egg
8 activation at fertilization of the newt *Cynops pyrrhogaster*. *Dev Biol* 306(2):797–808.
9 doi:10.1016/j.ydbio.2007.04.019
- 10 Haren L, Remy MH, Bazin I et al (2006) NEDD1-dependent recruitment of the γ -tubulin ring
11 complex to the centrosome is necessary for centriole duplication and spindle assembly. *J Cell*
12 *Biol* 172(4):505–515. doi:10.1083/jcb.200510028
- 13 Harpar EH (1904) The fertilization and early development of the pigeon's egg. *Am J Anat*
14 3(4):349–386
- 15 Homa ST, Swann K (1994) A cytosolic sperm factor triggers calcium oscillations and
16 membrane hyperpolarizations in human oocytes. *Hum Reprod* 9(12):2356–2361.
17 doi:10.1042/bj3130369
- 18 Hrabia A, Takagi S, Ono T et al (2003) Fertilization and development of quail oocytes after
19 intracytoplasmic sperm injection. *Biol Reprod* 69(5):1651–1657. doi:10.1095/
20 biolreprod.103.019315
- 21 Hughes RL, Hall LS (1998) Early development and embryology of the platypus. *Philos Trans*
22 *R Soc Lond B Biol Sci* 353(1372):1101–1114. doi:10.1098/rstb.1998. 0269
- 23 Hurst S, Howes EA, Coadwell J et al (1998) Expression of a testis-specific putative
24 actin-capping protein associated with the developing acrosome during rat spermiogenesis.
25 *Mol Reprod Dev* 49(1):81–91.
26 doi:10.1002/(SICI)1098-2795(199801)49:1<81::AID-MRD9>3.0.CO;2-Kdoi: 10.1002/(SICI)
27 1098-2795(199801)49:1<81::AID-MRD9>3.0.CO;2-K
- 28 Igusa Y, Miyazaki S, Yamashita N (1983) Periodic hyperpolarizing responses in hamster and
29 mouse eggs fertilized with mouse sperm. *J Physiol* 340:633–647.
30 doi:10.1113/jphysiol.1983.sp014784
- 31 Ito M, Shikano T, Oda S et al (2008) Difference in Ca^{2+} oscillation-inducing activity and
32 nuclear translocation ability of PLCZ1, an egg-activating sperm factor candidate, between

- 1 mouse, rat, human, and medaka fish. *Biol Reprod* 78(6):1081–1090.
- 2 doi:10.1095/biolreprod.108.067801
- 3 Iwamatsu T, Ohta T (1978) Electron microscopic observation on sperm penetration and
- 4 pronuclear formation in the fish egg. *J Exp Zool* 205(2):157–180. doi:10.1002/jez.1402050202
- 5 Iwao Y (1989) An electrically mediated block to polyspermy in the primitive urodele
- 6 *Hynobius nebulosus* and phylogenetic comparison with other amphibians. *Dev Biol*
- 7 134(2):438–445. doi:10.1016/0012-1606(89)90116-4
- 8 Iwao Y (2000) Fertilization in amphibians. In: Tarin JJ, Cano A (eds) *Fertilization in*
- 9 *Protozoa and Metazoan Animal*, Springer, Berlin, p 147–191
- 10 Iwao Y (2012) Egg activation in physiological polyspermy. *Reproduction* 144(1):11–22.
- 11 doi:10.1530/REP-12-0104
- 12 Iwao Y, Elinson RP (1990) Control of sperm nuclear behavior in physiologically polyspermic
- 13 newt eggs: possible involvement of MPF. *Dev Biol* 142(2):301–312.
- 14 doi:10.1016/0012-1606(90)90351-I
- 15 Iwao Y, Jaffe LA (1989) Evidence that the voltage-dependent component in the fertilization
- 16 process is contributed by the sperm. *Dev Biol* 134(2):446–451.
- 17 doi:10.1016/0012-1606(89)90117-6
- 18 Iwao Y, Murakawa T, Yamaguchi J et al (2002) Localization of γ -tubulin and cyclin B during
- 19 early cleavage in physiologically polyspermic newt eggs. *Dev Growth Differ* 44(6): 489–499.
- 20 doi:10.1046/j.1440-169X.2002.00661.x
- 21 Iwao Y, Sakamoto N, Takahara K et al (1993) The egg nucleus regulates the behavior of
- 22 sperm nuclei as well as cycling of MPF in physiologically polyspermic newt eggs. *Dev Biol*
- 23 160(1):15–27. doi:10.1006/dbio.1993.1282
- 24 Iwao Y, Yamasaki H, Katagiri C (1985) Experiments pertaining to the suppression of
- 25 accessory sperm in fertilized newt eggs. *Dev Growth Differ* 27(3):323–331.
- 26 doi:10.1111/j.1440-169X.1985. 00323.x
- 27 Jaffe LA, Gould M (1985) Polyspermy-preventing mechanisms. In: Metz CB, Monroy A
- 28 (eds) *Biology of Fertilization: The Fertilization Response of the Egg*, Academic Press.
- 29 Orlando, p 223-250
- 30 Jaffe LA, Sharp AP, Wolf DP (1983) Absence of an electrical polyspermy block in the mouse.
- 31 *Dev Biol* 96(2):317–323. doi:10.1016/0012-1606(83)90168-9

- 1 Jones KT (1998) Ca^{2+} oscillations in the activation of the egg and development of the embryo
2 in mammals. *Int J Dev Biol* 42(1):1–10
- 3 Jones KT (2005) Mammalian egg activation: from Ca^{2+} spiking to cell cycle progression.
4 *Reproduction* 130(6):813–823. doi:10.1530/rep.1.00710
- 5 Jones KT (2007) Intracellular calcium in the fertilization and development of mammalian
6 eggs. *Clin Exp Pharmacol Physiol*. 34(10):1084–1089. doi:10.1111/j.1440-1681.2007.04726.x
- 7 Jones KT, Carroll J, Merriman JA et al (1995) Repetitive sperm-induced Ca^{2+} transients in
8 mouse oocytes are cell cycle dependent. *Development* 121(10): 3259 –3266
- 9 Kang KS, Park TS, Rengaraj D et al. (2015) Fertilization of cryopreserved sperm and
10 unfertilized quail ovum by intracytoplasmic sperm injection. *Reprod Fertil Dev* in press.
11 doi:10.1071/RD15126
- 12 Keating TJ, Cork RJ, Robinson KR (1994) Intracellular free calcium oscillations in normal
13 and cleavage-blocked embryos and artificially activated eggs of *Xenopus laevis*. *J Cell Sci*
14 107(Pt 8): 2229–2237
- 15 Kobayashi W, Baba Y, Shimozawa T et al (1994) The fertilization potential provides a fast
16 block to polyspermy in lamprey eggs. *Dev Biol* 161(2):552–562. doi:10.1006/dbio.1994.1053
- 17 Kouchi Z, Fukami K, Shikano T et al (2004) Recombinant phospholipase $\text{C}\zeta$ has high Ca^{2+}
18 sensitivity and induces Ca^{2+} oscillations in mouse eggs. *J Biol Chem* 279(11):10408-10412.
19 doi:10.1074/jbc.M313 801200
- 20 Kuroda K, Ito M, Shikano T et al (2006) The role of X/Y linker region and N-terminal
21 EF-hand domain in nuclear translocation and Ca^{2+} oscillation-inducing activities of
22 phospholipase $\text{C}\zeta$, a mammalian egg-activating factor. *J Biol Chem* 281(38):27794–27805.
23 doi:10.1074/jbc.M603473200
- 24 Larman MG, Saunders CM, Carroll J et al (2004) Cell cycle-dependent Ca^{2+} oscillations in
25 mouse embryos are regulated by nuclear targeting of $\text{PLC}\zeta$. *J Cell Sci* 117(Pt 12):2513–2521.
26 doi:10.1242/jcs.01109
- 27 Lawrence Y, Ozil JP, Swann K (1998) The effects of a Ca^{2+} chelator and heavy-metal-ion
28 chelators upon Ca^{2+} oscillations and activation at fertilization in mouse eggs suggest a role for
29 repetitive Ca^{2+} increases. *Biochem J* 335(Pt 2):335–342. doi:10.1042/bj3350335
- 30 Lee B, Yoon SY, Malcuit C et al (2010) Inositol 1,4,5-trisphosphate receptor 1 degradation in
31 mouse eggs and impact on $[\text{Ca}^{2+}]_i$ oscillations. *J Cell Physiol* 222(1):238–247.
32 doi:10.1002/jcp.21945

- 1 Lee HC, Aarhus R, Walseth TF (1993) Calcium mobilization by dual receptors during
2 fertilization of sea urchin eggs. *Science* 261(5119):352–355. doi:10.1126/science.8392749
- 3 Leikin YN, Zharova TV, Tjulina OV (1993) Novel oxaloacetate effect on mitochondrial Ca^{2+}
4 movement. *FEBS Lett* 331(1-2):35–37. doi:10.1016/0014-5793(93)80292-3
- 5 Mahbub Hasan AK, Hashimoto A, Maekasa Y et al (2014) The egg membrane
6 microdomain-associated uroplakin III-Src system becomes functional during oocyte
7 maturation and is required for bidirectional gamete signaling at fertilization in *Xenopus Laevis*.
8 *Development* 141(8):1705–1714. doi:10.1242/dev.105510
- 9 Liu M (2011) The biology and dynamics of mammalian cortical granules. *Reprod Biol*
10 *Endocrinol* 9:149. doi:10.1186/1477-7827-9-149
- 11 Mahbub Hasan AK, Sato K, Sakakibara K et al (2005) Uroplakin III, a novel Src substrate in
12 *Xenopus* egg rafts, is a target for sperm protease essential for fertilization. *Dev Biol*
13 286(2):483–492. doi:10.1016/j.ydbio.2005.08.020
- 14 Malcuit C, Knott JG, He C et al (2005) Fertilization and inositol 1,4,5-trisphosphate
15 (IP3)-induced calcium release in type-1 inositol 1,4,5-trisphosphate receptor down-regulated
16 bovine eggs. *Biol Reprod* 73(1):2–13. doi:10.1095/biolreprod.104.037333
- 17 Marangos P, FitzHarris G, Carroll J (2003) Ca^{2+} oscillations at fertilization in mammals are
18 regulated by the formation of pronuclei. *Development* 130(7):1461–1472.
19 doi:10.1242/dev.00340
- 20 McAvey BA, Wortzman GB, Williams CJ et al. (2002) Involvement of calcium signaling and
21 the actin cytoskeleton in the membrane block to polyspermy in mouse eggs. *Biol Reprod*
22 67(4):1342–1352. doi:10.1095/biolreprod.102.004630
- 23 McCulloh DH, Rexroad CE Jr, Levitan H (1983) Insemination of rabbit eggs is associated
24 with slow depolarization and repetitive diphasic membrane potentials. *Dev Biol*
25 95(2):372–377. doi:10.1016/0012-1606(83)90038-6
- 26 Mehlmann LM, Carpenter G, Rhee SG et al (1998) SH2 domain-mediated activation of
27 phospholipase $\text{C}\gamma$ is not required to initiate Ca^{2+} release at fertilization of mouse eggs. *Dev*
28 *Biol* 203(1):221–232. doi:10.1006/dbio.1998.9051
- 29 Missiaen L, Parys JB, Smedt HD et al (1997) Effect of adenine nucleotides on myo-inositol-1,
30 4, 5-trisphosphate-induced calcium release. *Biochem J* 325 (Pt 3):661–666.
31 doi:10.1042/bj3250661

- 1 Miyagawa Y, Tanaka H, Iguchi N et al (2002) Molecular cloning and characterization of the
2 human orthologue of male germ cell-specific actin capping protein alpha3 (CPalpha3). *Mol*
3 *Hum Reprod* 8(6):531-593. doi:10.1093/molehr/8.6.531
- 4 Miyazaki S (1988) Inositol 1,4,5-trisphosphate-induced calcium release and guanine
5 nucleotide-binding protein-mediated periodic calcium rises in golden hamster eggs. *J Cell*
6 *Biol* 106(2):345–353
- 7 Miyazaki S (2006) Thirty years of calcium signals at fertilization. *Semin Cell Dev Biol*
8 17(2):233–243. doi:10.1016/j.semcdb.2006.02.007
- 9 Miyazaki S, Igusa Y (1981) Fertilization potential in golden hamster eggs consists of
10 recurring hyperpolarizations. *Nature* 290(5808):702–704. doi:10.1038/290702a0
- 11 Miyazaki S, Shirakawa H, Nakada K et al (1993) Essential role of the inositol
12 1,4,5,-trisphosphate receptor/ Ca^{2+} release channel in Ca^{2+} waves and Ca^{2+} oscillations at
13 fertilization of mammalian eggs. *Dev. Biol.* 158(1):62–78. doi:10.1006/dbio.1993.1168
- 14 Miyazaki S, Yuzaki M, Nakada K et al (1992) Block of Ca^{2+} wave and Ca^{2+} oscillation by
15 antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science*
16 257(5067):251–255. doi:10.1126/science.1321497
- 17 Mizushima S (2012) Establishment of intracytoplasmic sperm injection technique in Japanese
18 quail and its possible application for poultry resources and transgenic birds. *J Poult Sci*
19 49(4):225–230. doi:10.2141/jpsa.0120042
- 20 Mizushima S, Hiyama G, Shiba K et al (2014) The birth of quail chicks after intracytoplasmic
21 sperm injection. *Development* 141(19):3799–3806. doi:10.1242/dev.111765
- 22 Mizushima S, Takagi S, Ono T et al (2007) Possible role of calcium on oocyte development
23 after intracytoplasmic sperm injection in quail (*Coturnix japonica*). *J Exp Zool A Ecol Genet*
24 *Physiol* 307(11):647–653. doi:10.1002/jez.a.418
- 25 Mizushima S, Takagi S, Ono T et al (2008) Developmental enhancement of intracytoplasmic
26 sperm injection (ICSI)-generated quail embryos by phospholipase $\text{C}\zeta$ cRNA. *J Poult Sci*,
27 45(2): 152–158. doi:10.2141/jpsa.45.152
- 28 Mizushima S, Takagi S, Ono T et al (2009) Phospholipase $\text{C}\zeta$ mRNA expression and its
29 potency during spermatogenesis for activation of quail oocytes as a sperm factor. *Mol Reprod*
30 *Dev* 76(12): 1200–1207. doi:10.1002/mrd.21097

- 1 Mizushima S, Takagi S, Ono T et al (2010) Novel method of gene transfer in birds:
2 intracytoplasmic sperm injection for green fluorescent protein expression in quail blastoderms.
3 Biol Reprod 83(6):965-969. doi: 10.1095/biolreprod.110.085860
- 4 Moore GD, Kopf GS, Schultz RM (1993) Complete mouse egg activation in the absence of
5 sperm by stimulation of an exogenous G protein-coupled receptor. Dev Biol 159(2):669–678.
6 doi:10.1006/dbio.1993.1273
- 7 Mori M, Yamashita M, Yoshikuni M et al (1991) Maturation-promoting factor and p34cdc2
8 kinase during oocyte maturation of the Japanese quail. Dev Biol 146(1):246–249.
9 doi:10.1016/0012-1606(91)90465-F
- 10 Morito Y, Terada Y, Nakamura S et al (2005) Dynamics of microtubules and positioning of
11 female pronucleus during bovine parthenogenesis. Biol Reprod 73(5):935–941. doi:10.1095/
12 biolreprod.105.042366
- 13 Nagai K, Ishida T, Hashimoto T et al (2009) The sperm-surface glycoprotein, SGP, is
14 necessary for fertilization in the frog, *Xenopus laevis*. Dev Growth Differ 51(5):499–510.
15 doi:10.1111/j.1440-169X.2009.01112.x
- 16 Nakada K, Mizuno J, Shiraishi K et al (1995) Initiation, persistence, and cessation of the
17 series of intracellular Ca^{2+} responses during fertilization of bovine eggs. J Reprod Dev
18 41(1):77–84. doi:10.1262/jrd.41.77
- 19 Nakanishi A, Utsumi K, Iritani A (1990) Early nuclear events of in vitro fertilization in the
20 domestic fowl (*Gallus domesticus*). Mol Reprod Dev 26(3):217–221.
21 doi:10.1002/mrd.1080260304
- 22 Nuccitelli R (1980) The fertilization potential is not necessary for the block to polyspermy or
23 the activation of development in the medaka egg. Dev Biol 76(2):499–504.
24 doi:10.1016/0012-1606(80) 90397-8
- 25 Nuccitelli R, Kline D, Busa WB et al (1988) A highly localized activation current yet
26 widespread intracellular calcium increase in the egg of the frog, *Discoglossus pictus*. Dev
27 Biol 130(1):120–132. doi:10.1016/0012-1606(88)90419-8
- 28 Okamura F, Nishiyama H (1978) Penetration of spermatozoa into the ovum and
29 transformation of the sperm nucleus into the male pronucleus in the domestic fowl, *Gallus*
30 *gallus*. Cell Tissue Res 190(1):89–98. doi:10.1007/BF00210039

- 1 Olszanska B, Stepinska U, Perry MM (2002) Development of embryos from in vitro ovulated
2 and fertilized oocytes of the quail (*Coturnix coturnix japonica*). J Exp Zool 292(6): 580–586.
3 doi:10.1002/jez.10096
- 4 Ozil JP (1998) Role of calcium oscillations in mammalian egg activation: experimental
5 approach. Biophys Chem 72(1-2):141–152. doi:10.1016/S0301-4622(98)00130-6
- 6 Parrington J, Lai FA, Swann K (2000) The soluble mammalian sperm factor protein that
7 triggers Ca^{2+} oscillations in eggs: Evidence for expression of mRNA(s) coding for sperm
8 factor protein(s) in spermatogenic cells. Biol Cell 92(3-4):267–275.
9 doi:10.1016/S0248-4900(00)01064-9
- 10 Paterson JT (1910) Studies on the early development of the hen's egg. I. History of the early
11 cleavage and of the accessory cleavage. J Morphol 21(1):101–134
- 12 Payne C, Rawe V, Ramalho-Santos J et al (2003) Preferentially localized dynein and
13 perinuclear dynactin associate with nuclear pore complex proteins to mediate genomic union
14 during mammalian fertilization. J Cell Sci 116(Pt 23): 4727–4738. doi:10.1242/jcs.00784
- 15 Perry AC, Wakayama T, Cooke IM (2000) Mammalian oocyte activation by the synergistic
16 action of discrete sperm head components: induction of calcium transients and involvement of
17 proteolysis. Dev Biol 217(2):386–393. doi:10.1006/dbio.1999.9552
- 18 Perry AC, Wakayama T, Yanagimachi R (1999) A novel trans-complementation assay
19 suggests full mammalian oocyte activation is coordinately initiated by multiple, submembrane
20 sperm components. Biol Reprod 60(3):747–755. doi:10.1095/biolreprod60.3.747
- 21 Perry MM (1987) Nuclear events from fertilization to the early cleavage stages in the
22 domestic fowl (*Gallus domesticus*). J Anat 150:99–109
- 23 Quesada V, Sanchez LM, Alvarez J et al (2004) Identification and Characterization of human
24 and mouse ovastacin: a novel metalloproteinase similar to hatching enzymes from arthropods,
25 birds, amphibians, and fish. J Biol Chem 279(25):26627–26634. doi:
26 10.1074/jbc.M401588200
- 27 Rabbani MG, Sasanami T, Mori M et al (2006) Sperm-egg interaction is mediated by a
28 sperm-associated body in quail. Dev Growth Differ 48(1): 33–40.
29 doi:10.1111/j.1440-169X.2006.00842.x
- 30 Rabbani MG, Sasanami T, Mori M et al (2007) Characterization of the sperm-associated bdy
31 and its role in the fertilization of the chicken Gallus domestics. Dev Growth Differ
32 49(1):39–48. doi:10.1111/j.1440-169X.2007.00903.x

- 1 Reinsch S, Karsenti E (1997) Movement of nuclei along microtubules in *Xenopus* egg
2 extracts. *Curr Biol* 7(3):211–214. doi:10.1016/S0960-9822(97)70092-7
- 3 Rens W, O'Brien PC, Grutzner F et al (2007) The multiple sex chromosomes of platypus and
4 echidna are not completely identical and several share homology with the avian Z. *Genome*
5 *Biol* 8(11):R243. doi:10.1186/gb-2007-8-11-r243
- 6 Rhee SG (2001) Regulation of Phosphoinositide-Specific Phospholipase C. *Annu Rev*
7 *Biochem* 70:281–312. doi: 10.1146/annurev.biochem.70.1.281
- 8 Ridgway EB, Gilkey JC, Jaffe LF (1977) Free calcium increases explosively in activating
9 medaka eggs. *Proc Natl Acad Sci USA* 74(2):623–627
- 10 Roberts HE, Saxe DF, Muralidharan K et al (1996) Unique Mosaicism of tetraploidy and
11 trisomy 8: clinical, cytogenetic, and molecular findings in a live-born infant. *Am J Med Genet*
12 62(3):243–246.
13 doi:10.1002/(SICI)1096-8628(19960329)62:3<243::AID-AJMG8>3.0.CO;2-U
- 14 Ross PJ, Beyhan Z, Iager AE et al (2008) Parthenogenetic activation of bovine oocytes using
15 bovine and murine phospholipase C zeta. *BMC Dev Biol* 8:16. doi:10.1186/1471-213X-8-16
- 16 Rugh R (1951) The frog. Its reproduction and Development. *Yale J Biol Med* 23(5):441–442
- 17 Runft LL, Jaffe LA, Mehlmann LM (2002) Egg activation at fertilization: Where it all begins.
18 *Dev Biol* 245(2):237–254. doi:10.1006/dbio.2002.0600
- 19 Sakakibara K, Sato K, Yoshino K et al (2005) Molecular identification and characterization of
20 *Xenopus* egg uroplakin III, an egg raft-associated transmembrane protein that is
21 tyrosine-phosphorylated upon fertilization. *J Biol Chem* 280(15):15029–15037.
22 doi:10.1074/jbc.M410538200
- 23 Sakamoto I, Takahara K, Yamashita M et al (1998) Changes in cyclin B during oocyte
24 maturation and early embryonic cell cycle in the newt, *Cynops pyrrhogaster*: requirement of
25 germinal vesicle for MPF activation. *Dev Biol* 195(1):60–69. doi:10.1006/dbio.1997.8835
- 26 Sato K, Iwao Y, Fujimura T et al (1999) Evidence for the involvement of a Src-related
27 tyrosine kinase in *Xenopus* egg activation. *Dev Biol* 209(2):308–320.
28 doi:10.1006/dbio.1999.9255
- 29 Sato K, Ogawa K, Tokmakov AA et al (2001) Hydrogen peroxide induces Src family tyrosine
30 kinase-dependent activation of *Xenopus* eggs. *Dev Growth Differ* 43(1):55–72.
31 doi:10.1046/j.1440-169x.2001.00554.x

- 1 Sato K, Tokmakov AA, He CL et al (2003) Reconstitution of Src- dependent phospholipase
- 2 C γ phosphorylation and transient calcium release by using membrane rafts and cell-free
- 3 extracts from *Xenopus* eggs. J Biol Chem 278(40):38413–38420.
- 4 doi:10.1074/jbc.M302617200
- 5 Sato K, Tokmakov AA, Iwasaki T et al (2000) Tyrosine kinase-dependent activation of
- 6 phospholipase C γ is required for calcium transient in *Xenopus* egg fertilization. Dev Biol
- 7 224(2):453–469. doi:10.1006/dbio.2000.9782
- 8 Sato K, Wakai T, Seita Y et al (2013) Molecular characteristics of horse phospholipase C ζ
- 9 (PLC ζ). Anim Sci J 84(4):359–368. doi:10.1111/asj.12044
- 10 Saunders CM, Larman MG, Parrington J et al (2002) PLC ζ : a sperm-specific trigger of Ca²⁺
- 11 oscillations in eggs and embryo development. Development 129(15):3533–3544
- 12 Sett C, Paronetto MP, Barchi M et al (2002) Tr-kit-induced resumption of the cell cycle in
- 13 mouse eggs requires activation of a Src-like kinase. EMBO J 21(20):5386–5395.
- 14 doi:10.1093/emboj/cdf553
- 15 Sherard J, Bean C, Bove B et al (1986) Long survival in a 69XXY triploid male. Am J Med
- 16 Genet 25(2):307–312. doi:10.1002/ajmg.1320250216
- 17 Shimada K, Ono T, Mizushima S (2014) Application of intracytoplasmic sperm injection
- 18 (ICSI) for fertilization and Development in birds. Gen Comp Endocrinol 196:100–105.
- 19 doi:10.1016/j.ygcen.2013.11.001.
- 20 Shiono H, Azumi J, Fujiwara M et al (1988) Tetraploidy in a 15-month old girl. Am J Med
- 21 Genet 29(3):543–547. doi:10.1002/ajmg.1320290311
- 22 Snook RR, Hosken DJ, Karr TL (2011) The biology and evolution of polyspermy: insights
- 23 from cellular and functional studies of sperm and centrosomal behavior in the fertilized egg.
- 24 Reproduction 142(6):779–792. doi:10.1530/REP-11-0255
- 25 Srere PA (1992) The molecular physiology of citrate. Curr Top Cell Regul 33:261–75
- 26 Stepinska U, Olszanska B (2001) Detection of deoxyribonuclease I and II activities in
- 27 Japanese quail oocytes. Zygote 9(1):1–7. doi:10.1017/S0967199401001010
- 28 Stepinska U, Olszanska B (2003) DNase I and II present in avian oocytes: a possible
- 29 involvement in sperm degradation at polyspermic fertilisation. Zygote 11(1): 35–42.
- 30 doi:10.1017/S0967199403001059
- 31 Olszanska B, Stepinska U (2008) Molecular aspects of avian oogenesis and fertilization. Int J
- 32 Dev Biol 52(2-3):187–194. doi:10.1387/ijdb.072329ob

- 1 Stricker SA (1999) Comparative biology of calcium signaling during fertilization and egg
2 activation in animals. *Dev Biol* 211(2):157–176. doi:10.1006/dbio.1999.9340
- 3 Sultana F, Mao KM, Yoshizaki N (2004) Possible involvement of a sperm-associated body in
4 the process of fertilization in quail. *Zool Sci* 21(8):851–858. doi:10.2108/zsj.21.851
- 5 Sun QY (2003) Cellular and molecular mechanisms leading to cortical reaction and
6 polyspermy block in mammalian eggs. *Micro Res Tech* 61(4):342–348.
7 doi:10.1002/jemt.10347
- 8 Sun QY, Nagai T (2003) Molecular mechanism underlying pig oocyte maturation and
9 fertilization. *J Reprod Dev* 49(5):347–359. doi:10.1262/jrd.49.347
- 10 Swann K (1996) Soluble sperm factors and Ca^{2+} release in eggs at fertilization. *Rev Reprod*
11 1(1):33–39. doi:10.1530/ror.0.0010033
- 12 Swann K, Ozil JP (1994) Dynamics of the calcium signal that triggers mammalian egg
13 activation. *Int Rev Cytol* 152:183–222
- 14 Takagi S, Ono T, Tsukada A et al (2007a) Fertilization and blastoderm development of quail
15 oocytes after intracytoplasmic injection of chicken sperm bearing the W chromosome. *Poult*
16 *Sci* 86(5): 937–943. doi:10.1093/ps/86.5.937
- 17 Takagi S, Tsukada A, Saito N et al (2007b) Fertilizing ability of chicken sperm bearing the W
18 chromosome. *Poult Sci* 86(4):731–738. doi:10.1093/ps/86.4.731
- 19 Talevi R (1989) Polyspermic eggs in the anuran *Discoglossus pictus* develop normally.
20 *Development* 105:343–349
- 21 Tang TS, Dong JB, Huang XY et al (2000) Ca^{2+} Oscillations induced by a cytosolic sperm
22 protein factor are mediated by a maternal machinery that functions only once in mammalian
23 eggs. *Development* 127(5):1141–1150
- 24 Tatone C, Iorio R, Francione A et al (1999) Biochemical and biological effects of KN-93, an
25 inhibitor of calmodulin-dependent protein kinase II, on the initial events of mouse egg
26 activation induced by ethanol. *J Reprod Fertil* 115(1):151–157. doi:10.1530/jrf.0.1150151
- 27 Uchida IA, Freeman VC (1985) Triploidy and chromosomes. *Am J Obstet Gynecol*
28 151(1):65–69. doi:10.1016/0002-9378(85)90426-0
- 29 Ueno T, Ohgami T, Harada Y et al (2014) Egg activation in physiologically polyspermic newt
30 eggs: involvement of IP_3 receptor, $\text{PLC}\gamma$, and microtubules in calcium wave induction. *Int J*
31 *Dev Biol* 58(5):315–323. doi:10.1387/ijdb.130333yi
- 32 Van Krey HP, Ogasawara FX, Lorenz FW (1966) Distribution of spermatozoa in the oviduct

- 1 and fertility in domestic birds IV. Fertility of spermatozoa from infundibular and uteroviginal
- 2 glands. J Reprod Fertil 11(2):257–262. doi:10.1530/jrf.0.0110257
- 3 Waddington D, Gribbin C, Sterling RJ et al (1998) Chronology of events in the first cell cycle
- 4 of the polyspermic egg of the domestic fowl (*Gallus domesticus*). Int J Dev Biol
- 5 42(4):625–628
- 6 Waitzman JS, Rice SE (2014) Mechanism and regulation of kinesin-5, an essential motor for
- 7 the mitotic spindle. Biol Cell 106(1):1–12. doi:10.1111/boc.201300054
- 8 Wang WH, Machaty Z, Ruddock N et al (1999) Activation of porcine oocytes with calcium
- 9 ionophore: effects of extracellular calcium. Mol Reprod Dev 53(1): 99-107.
- 10 doi:10.1002/(SICI)1098-2795(199905)53:1<99::AID-MRD12>3.0.CO;2-G
- 11 Warren WC, Hillier LW, Marshall Graves JA et al (2008) Genome analysis of the platypus
- 12 reveals unique signatures of evolution. Nature 453(7192):175–183. doi:10.1038/nature06936
- 13 Wassarman PM (1994) Gamete interactions during mammalian fertilization. Theriogenology
- 14 41(1):31–44. doi:10.1016/S0093-691X(05)80046-9
- 15 Williams CJ, Mehlmann LM, Jaffe LA et al (1998) Evidence that Gq family G proteins do not
- 16 function in mouse egg activation at fertilization. Dev Biol 198(1):116–127.
- 17 doi:10.1006/dbio.1998.8892
- 18 Wishart GJ (1997) Quantitative aspects of sperm:egg interaction in chickens and turkeys.
- 19 Anim Reprod Sci 48(1):81–92. doi:10.1016/S0378-4320(97)00042-0
- 20 Wong JL, Wessel GM (2006) Defending the zygote: search for the ancestral animal block to
- 21 polyspermy. Curr Top Dev Biol 72:1–151. doi:10.1016/S0070-2153(05)72001-9
- 22 Wu H, He CL, Fissore RA (1997) Injection of a porcine sperm factor triggers calcium
- 23 oscillations in mouse oocyte and bovine eggs. Mol Reprod Dev 46(2):176–189.
- 24 doi:10.1002/(SICI)1098-2795(199702)46:2<176::AID-MRD8>3.0.CO;2-N
- 25 Wu H, He CL, Jehn B et al (1998) Partial characterization of the calcium-releasing activity of
- 26 porcine sperm cytosolic extracts. Dev Biol 203(2):369–381. doi:10.1006/dbio.1998.9070
- 27 Yanagimachi R (1994) Mammalian fertilization. In: Knobil E, Neill JD (eds) The Physiology
- 28 of Reproduction, 2nd edn. Raven Press, New York, p 189–319
- 29 Yanagimachi R (2005) Intracytoplasmic injection of spermatozoa and spermatogenic cells: its
- 30 biology and applications in humans and animals. Reprod Biomed Online 10(2):247–288.
- 31 doi:10.1016/S1472-6483(10)60947-9

- 1 Yoda A, Oda S, Shikano T, Kouchi Z et al (2004) Ca^{2+} oscillation-inducing phospholipase C
2 zeta expressed in mouse eggs is accumulated to the pronucleus during egg activation. *Dev*
3 *Biol* 268(2):245–257. doi:10.1016/j.ydbio.2003.12.028
- 4 Yoneda A, Kashima M, Yoshida S et al (2006) Molecular cloning, testicular postnatal
5 expression, and oocyte-activating potential of porcine phospholipase C ζ . *Reproduction*
6 132(3):393–401. doi:10.1530/rep.1.01018
- 7 Yoshimura Y, Tanaka H, Nozaki M et al (1999) Genomic analysis of male germ cell-specific
8 actin capping protein alpha. *Gene* 237(1):193–199. doi:10.1016/S0378-1119(99)00287-5
- 9 Young C, Grasa P, Coward K et al (2009) Phospholipase C zeta undergoes dynamic changes
10 in its pattern of localization in sperm during capacitation and the acrosome reaction. *Fertil*
11 *Steril* 91(5 Suppl):2230–2242. doi:10.1016/j.fertnstert.2008.05.021
- 12 Xia P, Wang Z, Yang Z et al (2001) Ultrastructural study of polyspermy during early embryo
13 development in pigs, observed by scanning electron microscope and transmission electron
14 microscope. *Cell Tissue Res* 303(2):271–275. doi:10.1007/s004410000315
- 15 Zhu CC, Furuichi T, Mikoshiba K et al (1999) Inositol 1,4,5-trisphosphate receptor
16 down-regulation is activated directly by inositol 1,4,5-trisphosphate binding. Studies with
17 binding-defective mutant receptors. *J Biol Chem* 274(6):3476–3484.
18 doi:10.1074/jbc.274.6.3476
- 19 Zhu CC, Wojcikiewicz RJ (2000) Ligand binding directly stimulates ubiquitination of the
20 inositol 1,4,5-trisphosphate receptor. *Biochem J* 248(Pt 3):551–556. doi:10.1042/bj3480551
21

Figure Captions

Fig.7.1. Scanning electron microscopic observation of two sperm and egg plasma membrane fusion in Japanese quail (photograph provided by Dr. Norio Yoshizaki, Gifu University). Usually, 2-60+ sperm penetrate egg cytoplasm in birds.

Fig.7.2. Ca^{2+} rise in quail egg cytoplasm after microinjection of sperm-derived egg activating factors. An intracellular Ca^{2+} increased immediately from injecting site and the Ca^{2+} signal propagated over the egg cytoplasm. The Ca^{2+} rise continues for about 5 minutes. Thereafter, long-lasting spiral-like Ca^{2+} oscillations are generated and continue every 1 min for over 1 hour. Small circle indicates injecting site.

Fig.7.3. Schematic signaling pathway in avian egg activation. Sperm-specific $\text{PLC}\zeta$ introduced from each sperm induces a slow Ca^{2+} wave, while sperm-specific CS and AH induces a periodic spiral-like Ca^{2+} spike. Inositol 1,4,5-trisphosphate (IP_3) hydrolyzed from phosphatidylinositol 4,5-bisphosphate by $\text{PLC}\zeta$ activity evokes Ca^{2+} release from endoplasmic reticulum (ER) via IP_3 receptor. CS and AH may induce Ca^{2+} release from ER or mitochondria. In a part, another molecules and ryanodin receptor may be involved in Ca^{2+} release.

Fig.7.4. Ca^{2+} rise and zygote formation in avian egg cytoplasm. (A) At immediate after sperm penetration, Ca^{2+} waves spreads in the whole egg cytoplasm from each sperm entry site. (B) At 15 min, a spiral-like Ca^{2+} spikes from each sperm entry site are generated and it continues to pronuclear stage. (C) At 1.5 hours, the female chromatin is in metaphase II (MII) of the second meiosis and the incorporated sperm are starting to transform to pronuclei in order of entry into egg cytoplasm. (D) At 3 hours, female (FPN) and principal male pronucleus (PMPN) places in the center of egg cytoplasm, whilst supernumerary sperm pronuclei (SPN) move towards the in the vicinity of cytoplasm. (E) At 4.0 hours, the zygotic nucleus and several supernumerary sperm nuclei undergo mitosis. (F) At 5 hours, a zygotic nucleus progresses towards 4-cell stages and supernumerary sperm are degraded by DNases. Modified from J Anat 150:99-109 (Perry 1987).

1 Table 8.1 Comparison of fertilization mode, pattern of Ca^{2+} rise in egg activation, and
 2 sperm-derived egg-activating factor among vertebrates

Species	Fertilization mode	Ca^{2+} pattern	Ca^{2+} store	Sperm factor
fish				
Fugu	monospermy	single	endoplasmic reticulum	?
medaka	monospermy	single	endoplasmic reticulum	$\text{PLC}\zeta$?
<i>Discoglossus</i>	polyspermy	multiple	endoplasmic reticulum	?
amhibian				
anuran (<i>Xenopus</i>)	monospermy	single	endoplasmic reticulum	receptor
anuran (<i>Discoglossus</i>)	polyspermy	?	?	?
urodele (<i>Cynopus</i>)	polyspermy	multiple	endoplasmic reticulum, mitochondria?	CS
bird				
Japanese quail	polyspermy	multiple	endoplasmic reticulum, mitochondria?	$\text{PLC}\zeta$, CS, AH
chicken	polyspermy	?	?	$\text{PLC}\zeta$?
mammal				
platypus	polyspermy	?	?	?
higher eutherians	monospermy	multiple	endoplasmic reticulum	$\text{PLC}\zeta$

Mizushima Figure 7.1

mizushima Figure 7.2

Mizushima Figure 7.3

(A) Ca^{2+} wave

(B) Spiral-like Ca^{2+} oscillation

(C) Transformation

(D) Pronuclear stage

(E) First cleavage stage

(F) 4-cell stage

Mizushima Figure 7.4