4

FERTILIZATION

Investigations on fertilization date back before the turn of the century under the leadership of cytologists such as **Van–Beneden, Flemming, Strasburger, Boveri** and **Wilson** whose observations on germ cells were closely affiliated with theoretical writings of **Nageli, Weissman, Hertwig, Roux** and **De Vries** (Wilson, 1925).

Fertilization is a complex process involving the fusion of male and female gametes followed by the fusion of their cytoplasm and **nuclei resulting—biparental heredity. Manner Harold** (1964) defines fertilization **as the entire process beginning with the sperm's approach to egg and ending with the fusion of the egg and sperm pronuclei.** In this event all the strands of the webs of two lives are gathered in one knot, from which a rejuvenated new individual is formed. The main idea of fertilization was enunciated by **Leeuwenhock** (1962). Essentially, the process of fertilization has two independent **significance** or **functions**—the **activation** and **amphimixis.**

1. Activation involves—(*i*) the completion of second maturation division, if not occurred earlier; (*ii*) the egg cortex to form fertilization membrane outside the plasma membrane; (*iii*) the egg cytoplasm to start various metabolic reactions; (*iv*) stimulation of mitosis for cleavage by the contribution of sperm's centriole to the egg.

2. Amphimixis or the fusion of the sperm and egg nuclei involves—(*i*) The intermingling of paternal and maternal hereditary characters in the offspring (mixing of genes); (*ii*) restoration of diploid state of animal cell; (*iii*) induction of genetic variations in the species. Which provides raw material to natural selection.

The **fertilizability** and **viability** of **gametes** are limited and variable. Fertilization requires fluid medium, either water for aquate forms or some body fluid in viviparous animals. The eggs that are shed into water must be fertilized immediately within a few minutes. However, the eggs that are fertilized within the body of the female generally have a longer span. In *Styela*, a urochordate, the egg may remain alive and capable of fertilization for 3 to 4 hours after it is discharged into the sea-water. In *Fundulus* and many other teleost fishes, the egg retains fertility only for 15 to 20 minutes. In frog, under normal conditions, the egg remains alive for 3 to 5 days without producing any abnormalities. Among mammals, the viability of egg after ovulation, varies considerably. In lower mammals this period is brief and is a matter of hours rather than days. In mare and rabbit, fertilization must occur within about 2 to 4 hours; rat 10 hours and mouse 12 to 14 hours. Similarly, the monkey becomes pregnant only when mated just before the time of ovulation. For the human ovum, it is generally believed that the fertilizable period is 24 hours after ovulation.

If a mammalian egg does not become fertilized, it begins to degenerate while still in the oviduct. In guinea pig a functional decline enters as early as eight hours after ovulation. The egg becomes unfertilizable after 20 hours and enters a state of physical deterioration within 24 hours. When spermatozoa are discharged into genital tract of female mammal, they immediately

encounter hazards to their longevity and loses ability to fertilize female gamete long before it loses motility. The ingestion of spermatozoa by leucocytes and acidity of the vagina is fatal. They also undergo a rapid decline in vigour because of their limited amount of potential energy.

The fertilizing span of sperms within the genital tract of female mammals varies between six hours (mouse) to six days (mare). In rabbit, the sperms are in viable condition for about 10 to 14 hours, while in bat it may persist in the female reproductive tract over the winter. The hen retains functional sperms in its oviducts for about 3 weeks, while a period of four years has been claimed in *Terrapins*. The use of frozen spermatic fluid in the delayed artificial insemination of cattle is now, a standard practice. Though the life span in the female tract is 24 hours but human semen, frozen in glycerol at – 70° C, yields high mobility in surviving spermatozoa after many months of storage.

To ensure the maximum probability of fertilization, the **number** of sperms must exceed the number of eggs. Thus, in mammals, a single ejaculation may range from 0.05 ml in bat to 2 ml in rat to 500 ml in bear containing 6 millions, 2 to 5 millions, and 100,000 cells per microlitre respectively. In man, the ejaculate averages 3 ml containing about 100,000 cells per microlitre.

Viability of sperms may be affected by nutritional factors, affecting the male animal by weakening the sperm cells. Deficiency of vitamin E destroy the fertilizing power of sperms. Extremes of heat and cold injurious to developing germ cells. Sperms are also sensitive to the pH of the medium, can withstand considerable degree of alkalinity but slight acid condition is fatal. In mammals, the acidity of the vagina is a common cause of sterlity.

Mechanism of fertilization

The mechanism of fertilization includes those consecutive steps, which leads to fertilization of the egg and the changes occurring in the egg during and after fertilization :

Major steps are :

1. Approach of spermatozoa to ova.
2. Capacitation and fertilizin–antifertilizin reaction.
3. Acrosomal reaction and penetration of sperm.
4. Activation of ovum.
5. Spermatozoan in the egg interior, migration of pronuclei and amphimixis.

1. Approach of spermatozoa to ova

A major problem in sexual reproduction is how to bring together the spermatozoa and ova in the same locality so that individual sperm may reach the surface of ova at the right time. According to the place and nature of fluid media, following two kinds of fertilization have been reported :

External fertilization. The type of fertilization occurs in liquid medium outside the bodies of parent animals. Among fresh water animals (fishes, amphibians and fresh water invertebrates), the sperms are delivered directly to the eggs immediately after egg laying, because their spermatozoa remain active usually for a few minutes. These animals exhibit **pseudocopulation** or **amplexus.** But marine forms like sea-urchins, shed eggs and sperms freely into the surrounding water. The adult members of a species usually become sexually mature depending upon the environmental temperatures of the preceding weeks or months. One or more ripe female spawn at dawn or dusk, stimulated by the rapid change in light intensity. Substances liberated with the oviducal fluid at the time of shedding of eggs in turn stimulate other ripe females and males in the vicinity. Consequently clouds of eggs and sperms are seen in the water at the same time, and mass fertilization occurs. Even then, to reach the eggs, spermatozoa have to travel long distance and their chances of encounter with the eggs may greatly scared. The time interval between the laying of eggs and the shedding of sperms may even be weeks or months, but the saltish sea water serves an important physiological medium for gametes.

Internal fertilization. In terrestrial oviparous forms (such as reptiles and birds), the eggs are completely enclosed in impermeable egg membranes or they are retained within the maternal body throughout development in ovoviviparous and viviparous animals, the spermatozoa are delivered internally in the body of the female by some type of copulatory mechanism or by intromittent organ of the male. In such forms the fertilization may occur in the lower part of the oviduct (*e.g.*, Urodela); in the upper portion of oviduct (*e.g.*, salamanders, reptiles, birds and mammals) or in the ovarian follicles in viviparous fishes (*e.g.*, *Gambusia affinis*) and eutherian mammals (*e.g.*, *Ericulus*). The sperm reaches near the ovum by either of the two strategies.

(*i*) Chaemotaxis

A chemical substance, the **fertilizin** secreted from the cortical region of egg cytoplasm of sea urchin egg was supposed to attract the sperms towards egg. The evidence as to weather any fertilizin is present in the egg or its surface is very contradictory (**Metze,** 1957). The swimming movement of spermatozoa in a liquid media, is entirely at random and the spermatozoa collide with the eggs as a matter of chance partly due to enormous number of spermatozoa produced by the male and partly because the eggs being relatively large target, so that it can be hit fairly well.

Frank Lillie showed that a chemical substance is discharged by the newly laid sea-urchin egg. This substance diffuses into the water and causes sperm cells in the neighbourhood to become more active and being attracted to the egg of the same species. The condition would be termed **chaemotaxis,** since the sperms were responding to this chemical substance. **Tylar** has found this substance in the jelly which surrounds the egg. Similarly, a chemical sperm attractant is produced by fish egg.

Sperm chaemotaxis disappears following fertilization possibly as a result of cortical granule exocytosis. Although the involvement of other egg changes have not been eliminated.

(*ii*) Mechanical Juxtaposition of gametes.

In viviparous fish, *Gambusia affinis* and eutherian mammal, *Ericulus*, the sperms are deposited in the vagina. The movement of the spermatozoa from the site of deposition to the site of fertilization usually depends on the active swimming of the spermatozoa themselves in body fluid or transported passively by muscular contractions of the female tract and also by the counter currents in the cilia which propel backward flowing liquid content of the tract.

2. Capacitation

Only morphological differentiation of mammalian secondary spermatocytes into spermatids, during the process of second maturation division is not sufficient to make them capable of fertilizing an ovum. Spermatozoa removed from the seminiferous tubules of testis, caput or cauda epididymis or even from the vas deferens, are also not capable of fertilizing egg though they are motile in the later case. Some elaborate and essential changes seem to occur in the spermatozoa from the time of their release into the seminiferous tubules until they reach the vicinity of egg in the upper part of oviduct in the process of copulation where they are being exposed to the influence of the fluids in the reproductive tract of the female. The spermatozoa become capable of penetrating through the egg membranes and fusing with the egg.

The changes in mammalian spermatozoon which make it capable of fertilizing the egg have been called Capacitation. In other words, **the capacitation may result from the removal of some inhibiting material from the sperm.** These changes were first recognized by **Austin** (1951) and **Chang** (1951). The essential requirements of a few hours residence of the sperm in the female reproductive tract before acquiring the ability to fertilize was demonstrated by **Farooqui** (1983) and **Bedford** (1983). The phenomenon of capitation relates to the specificity of fertilization, *i.e.*, the capacity of spermatozoa to fertilize eggs of the same species but not of others.

Acrosome reaction is a structural change that finally renders the sperm ready for fertilization. Capacitation and acrosome reaction are closely related processes which go hand in hand. According to **Bavister** (1986) capacitaion is to acquire the ability to undergo acrosome reaction.

One of the physiological correlates of capacitation is a peculiar **'Whiplash'** type of sperm motility known as **hyperactivation** exhibited by hamster and rabbit spermatozoa within the oviducal ampulla. Hyperactivation is also associated with the process of capacitation of mouse, bat, dog, guinea pig, marmoset monkey and bovine spermatozoa. Hamster epididymal spermatozoa, if incubated in a culture medium containing bovine serum albumen (BSA), show hyperactive motility and are capacitated as indicated by their ability to undergo acrosome reaction. In the absence of BSA, the sperm shows hyperactivity but are not capacitated (**Bavister,** 1986)

Capacitation, however, involves *molecular changes* in the sperm's plasma membrane, the breaking up of the membranes surrounding the acrosome and the release of the contents of acrosome, consisting of sperm lysins. Sperm lysins aid the spermatozoon in penetrating the layers of follicle cells and the zona pellucida (**Bedford,** 1967) in mammals.

Sperm acquires surface coatings from epididymal secretions. Proteins derived from seminal plasma bind firmly on the sperm surface, which form major part of sperm plasma membrane protein (**Russel et. al.** 1984). Removal of these surface coating from the sperm is essential for capacitation. However, capacitation is not merely a loss of surface substances because sperm before acquiring them also cannot fertilize efficiently.

Much of the information with regard to the process of capacitation had been obtained from *in vitro* studies. A Con–A binding pattern on rabbit spermatozoa obtained from the male and female genital tracts has revealed the complex nature of capacitation changes. Caput-sperm shows very little Con-A binding whereas the cauda-sperm exhibited uniform dense binding. As indicated that lectin binding sites develop during the transit from caput to cauda epididymis. Sperm from semen before deposition in the female gential tract shows no binding. This is because of masking of the binding sites by some components of seminal fluids. Capacitated sperm obtained after its deposition in the female genital tract, exhibited reduced binding over the acrosome but retained the dense binding pattern over the post-acrosomal surface. The changing pattern of Con-A binding possibly reflects surface changes relevant to the recognition of the varying environment through which the sperm pass.

Capacitation time is about six hours for rabbit and one hour for mice. Human sperms must be in the female reproductive tract for about seven hours before they can fertilize eggs. Capacitation apparently involves removing deactivating or decapacitating factor that binds to sperms as they pass through the male reproductive tract. This factor apparently block the acrosome reactions.

In vitro studies have revealed a number of factors that induce as well inhibit capacitation :

(*i*) pH is an important factor controlling capacitation. Low pH (= 6.1) is inhibitory to capacitation of hamster and dog sperms. At higher pH (= 7.4) capacitation can occur. However, it has been suggested that the $HCO_3 - CO_2$ system may be the *in vivo* mechanism of p H regulation in controlling capacitation.

(*ii*) The ratio of Na^+/K^+ is also involed in inducing capacitation. A high Na^+/K^+ ratio obtained in the secretions of uterus, isthmus and ampulla permit capacitation.

(*iii*) A substantial amount of Zn ions associated with mammalian sperms appear to inhibit sperm capacitation while serum albumin is known to bring about capacitation.

(*iv*) In most *in vitro* studies, bovine serum albumin is routinely included in culture media for obtaining acrosome reaction in fertilization. Though a requirement of serum albumin in capacitation of golden hamster and mouse spermatozoa has been demonstrated. But in the

case of human, serum albumin is not essential for capacitation. Although serum albumin in the secretions of the gential tract may play a positive role, but its exact mode of action is not clear.

As the capacitation changes are essentially restricted to the sperm surface, yet they are functionally related to acquiring the ability to respond to the zona stimulus and to undergo acrosome reaction.

Invertebrate sperms transferred to females for storage in seminal receptacles have been shown in some cases to undergo maturational changes similar to those occurring in the capacitation of mammalian sperm. Such changes have been demonstrated in shrimps, *Siconia* (**Clark et. al.,** 1984). Seminal fluid of the sea-urchin, *Arbacia*, prevents rapid metabolic decline of sperm. If sperms are added to egg jelly in the presence of seminal fluid, respiration and viability are prolonged. This may be a result of surface modification so that the activity of the spermatozoon is altered (**Shapiro** and **Eddy,** 1980). This process is similar to capacitation in mammals.

In the end it may be concluded that (*i*) the phenomenon of capacitation relates to the specificity of fertilization *i.e.*, capacity of spermatozoa to fertilize eggs of the same species but not of others ; (*ii*) Capacitation, a hyper-activation and acrosome reaction are preparatory changes undergo by the sperm before fusing with the egg ; (*iii*) Sperm capacitation occurs in a few hours when the sperm suspension is stored at room temperature; *(iv)* The aerosomes of the human sperm contain powerful hydrolytic and proteolytic enzymes that could destroy the mare genital tract if all the sperms could release these enzymes prematurely. But the fluids in seminiferous tubules, in the epididymis and in the vas-deferens contain minute floating vesicles filled with large amount of cholesterol. This cholesterol is continuously donated to the cellular membrane covering the acrosome, toughening this membrane and preventing the release of the enzymes. After ejaculation, sperms swim away from the vesicles and gradually lose their excess cholesterol during the next few hours. In so doing they become **"capicitated"** so that the acrosome can now release the enzymes that allow a sperm to enter the ovum (**Guyton,** 1986).

(*a*) Agglutination. In most animals (polychaetes, molluscs, echinoderms, tunicates and even vertebrates), it has been observed that the spermatozoa become adhere to the surface of the egg by their lateral aspect, and even to each other. This reaction is usually visible within a few seconds of the release of chemical substance and the spermatozoa are seen to clump together head to head or less commonly tail to tail. The adhesion of the spermatozoa results in their **clumping** or **agglutination.** It depends to a large extent on the environmental conditions. Agglutination of the spermatoza to the ova of the same species has also been widely studied in most animals.

(*b*) Fertilizin-antifertilizin reaction. The cause of agglutination of spermatozoa in higher animals especially the vertebrates depends to a large extent on the fertilizin and antifertilizin reaction, which was studied by **Lillie** (1919). These reacting substances **fertilizin** and **antifertilizin** are continuously emitted from mature eggs and sperms respectively. Both these species-specific chemical molecules form a sort of chemical lock-up like an antigen and an antibody. The main source of fertilizin is the egg itself and remain located in plasma membrane. However, in the eggs of sea-urchin and other echinoderms, it is produced by the layer of jelly surrounding the egg, and becomes accumulated in the external gelatinous coat. In mammals, the cells of zona pellucida produce fertilizin.

The fertilizin is a gel formed of glycoprotein or mucopolysaccharide. As a protein, it contains a number of amino acids and as a polysaccharides it includes molecules of one or more monosaccharide. The monosaccharides are esterified by sulphuric acid. Both the monosaccharides and amino acids of fertilizin vary from one species to another, that is why, each

species possesses its specific type of fertilizin. Fertilizin makes the sperm sticky so that they adhere into clumps. The molecular weight of fertilizin is 30,000.

The antifertilizin is acid proteins contained in the surface layer of the sperm cytoplasm (*i.e.*, sperm plasma membrane). Its molecular weight is 10,000.

These molecules of the two gametes are specific interacting substances. The remarkable peculiarity of fertilizin and antifertilizin is that they combine in a specific way. Each molecule of fertilizin may have more than one "active group", so that one fertilizin particle may become attached with two or more molecules of antifertilizin of sperm of the same species (Fig. 4.1). Thus, during the capacitation and contact stage of fertilization, when spermatozoa and eggs of the same species come in physical contact with each other, a chemical lock up is established between the antifertilizin molecules of spermatozoa and fertilizin molecules of unfertilized egg. Due to this fact many spermatozoa adhere to the surface of an unfertilized egg and ensures fertilization.

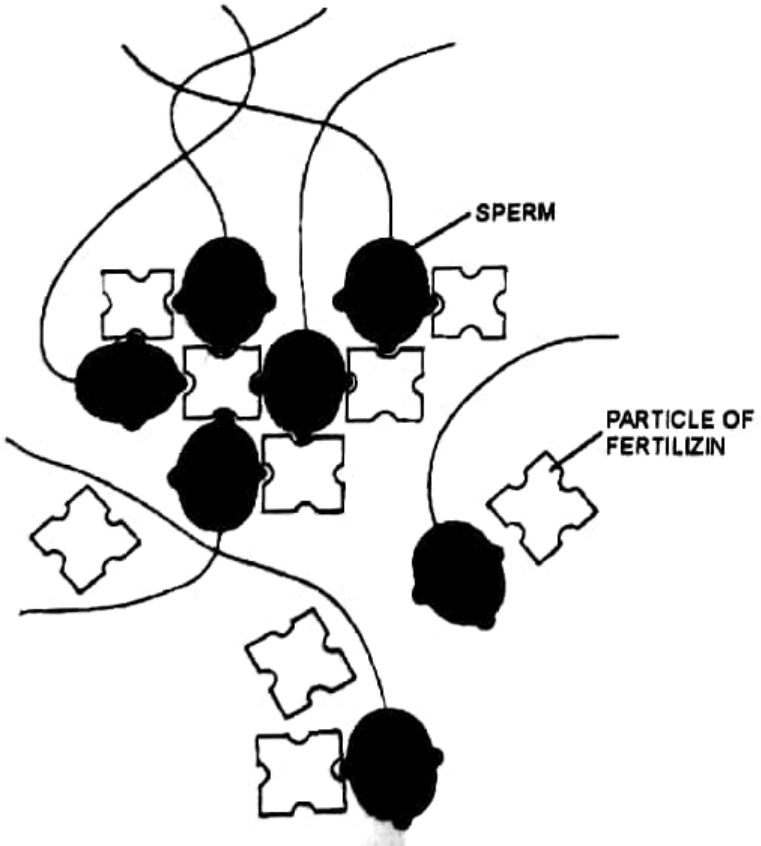

Fig. 4.1. Agglutination of sperm (after Balinsky, 1976).

The main function of the fertilizin-antifertilizin reaction is to thin out the number of spermatozoa around the egg, so that the chances of two or more spermatozoa penetrating into the egg at the same time are diminished (**Runnstrom and** others, 1959).

1. Importance of Fertilizin-Antifertilizin reaction

(*i*) The sperms identify the egg by fertilizin-antifertilizin reaction.

(*ii*) The initial attachment of the sperm to the egg is the result of the linking between fertilizin particles and antifertilizin particles.

(*iii*) Certain amount of fertilizin released from the egg into the surrounding water. These released fertilizins combine with the sperms. This leads to the agglutination of sperms. As a result of this only a few sperms react to surface of the egg. This prevents polyspermy.

(*iv*) As the reaction between fertilizin and antifertilizin is species specific, fertilization between different species is prevented.

(*v*) Sperms contain lytic substances which can break down the egg coat. By holding together many sperms on the egg surface, the fertilizin-antifertilizin reaction ensures the production of sufficient quantities of lytic enzymes to dissolve the egg membranes.

(*vi*) Fertilizin activates the sperms and initiates acrosomal reactions.

2. Acrosome reaction and penetration of sperm

Except those of coelenterates, most animal eggs are enveloped by one or more egg membranes or gelatinous layers or follicle cells or both, outside the plasma membrane. These layers not only constitute barriers for the penetration by spermatozoa and serve in preventing fertilization by more than one spermatozoon or by sperm of other species.

When a spermatozoon is attached with the surface of the egg, it becomes motionless. Its penetration through egg membranes and also

through the plasma membrane of the egg is achieved by some physicochemical activity of the sperm acrosome :

(*i*) Certain enzymatic proteins called **sperm lysins** are produced presumably by the sperm acrosome (**Colwin** and **Colwin,** 1961). In certain invertebrates, those enzymes dissolve the egg membranes locally and make the path clear for spermatozoon to reach the surface of the egg.

(*ii*) In the eggs of insects, nemertines, gastropods, pelecypods, cephalopods, echinoderms and fishes which have thick and resistant membranes, and sperm cannot reach the egg at all points except through the micropyle or canal, left in the egg membrane.

(*iii*) In mammals, the eggs are encased in a layer of follicular cells, called **corona radiata.** Follicular cells are held together by a cementing substance called **hyaluronic acid,** which is a mucopolysaccharide. The corona radiata acts as barrier through which the spermatozoon must first penetrate to reach the plasma membrane of the egg. To do so the sperm's acrosome produces an enzyme, **hyaluronidase,** which serves to dissolve the adhesive substance and disperse the cells of corona radiata. Moreover, the acrosome itself undergoes morphological changes and forms **acrosomal filament** in *saccoglossus* which helps the sperm penetration into the egg interior (**Colwin** and **Colwin,** 1967).

The activated spermatozoon on reaching the egg plasma membrane undergoes a number of changes in its acrosomal region. All these changes are collectively described under **acrosome reaction.**

A spermatozoon of *Saccoglossus* has spherical nucleus, an acrosomal vesicle at the forward end of the sperm head and a long, flat tail. The acrosomal vesicle is bounded by an

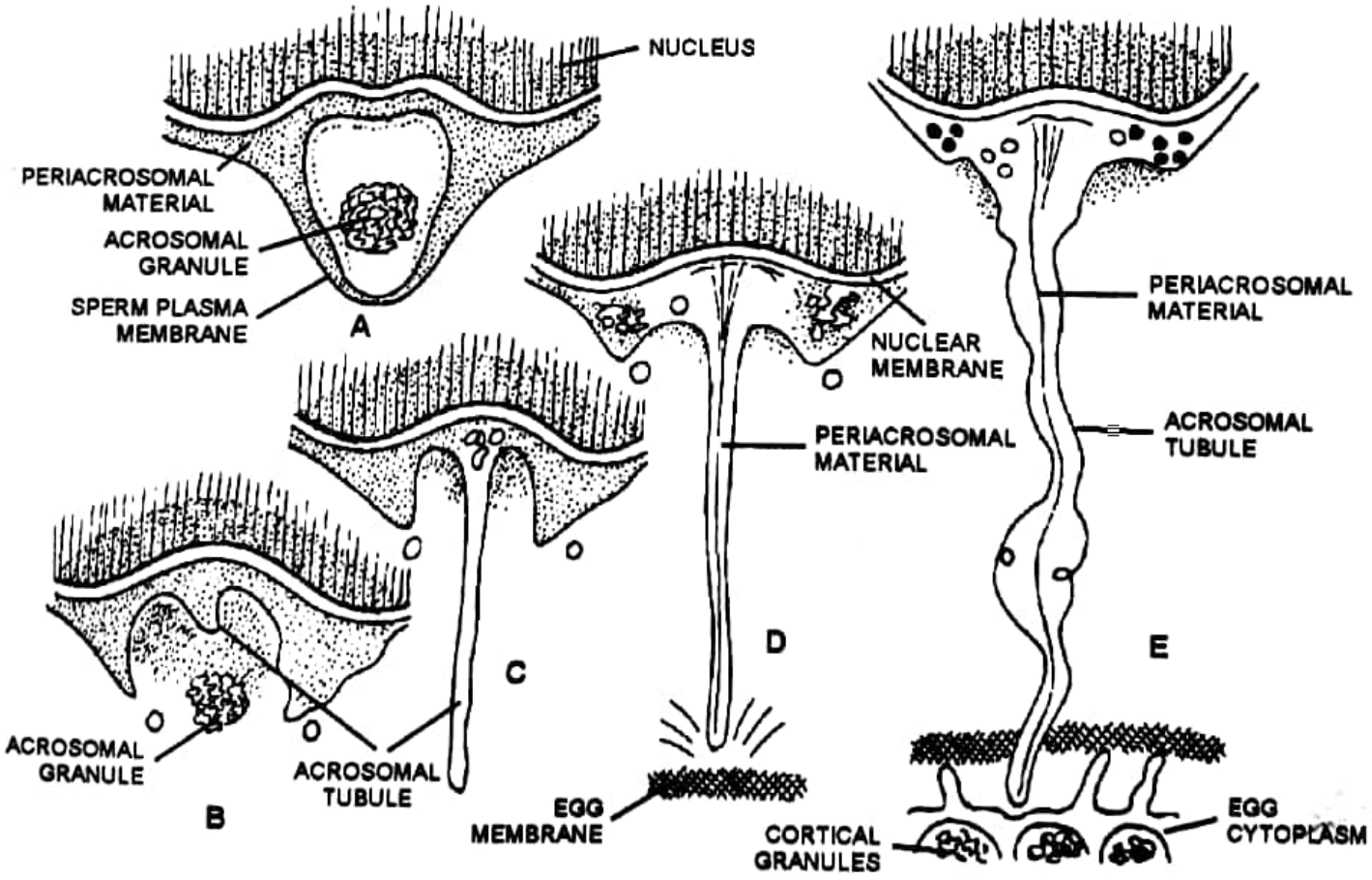

Fig. 4.2. Changes in the spermatozoon during fertilization in *Saccoglossus*. A – Acrosome of inactivated spermatozoon; B – Extrusion of acrosomal granules; C – Formation of acrosomal tubule; D – Acrosomal tubule reaches egg membrane; E – Acrosomal filament reaches the surface of egg cytoplasm after (Balinsky, 1976).

acrosomal membrane and contains a large, dense **acrosomal granule** (Fig. 4.2). The entire granule is surrounded by fine granular material except at the apex where an apical space is present between the granule and a membrane. The space between the acrosomal membrane and the sperm plasma membrane as well, the space between acrosomal membrane and nuclear membrane, are filled by some material called perforosomal material. As soon as the spermatozoon of *Saccoglossus* makes its initial contact with the egg surface, following events set in :

(*i*) The apex of the acrosome burst so that

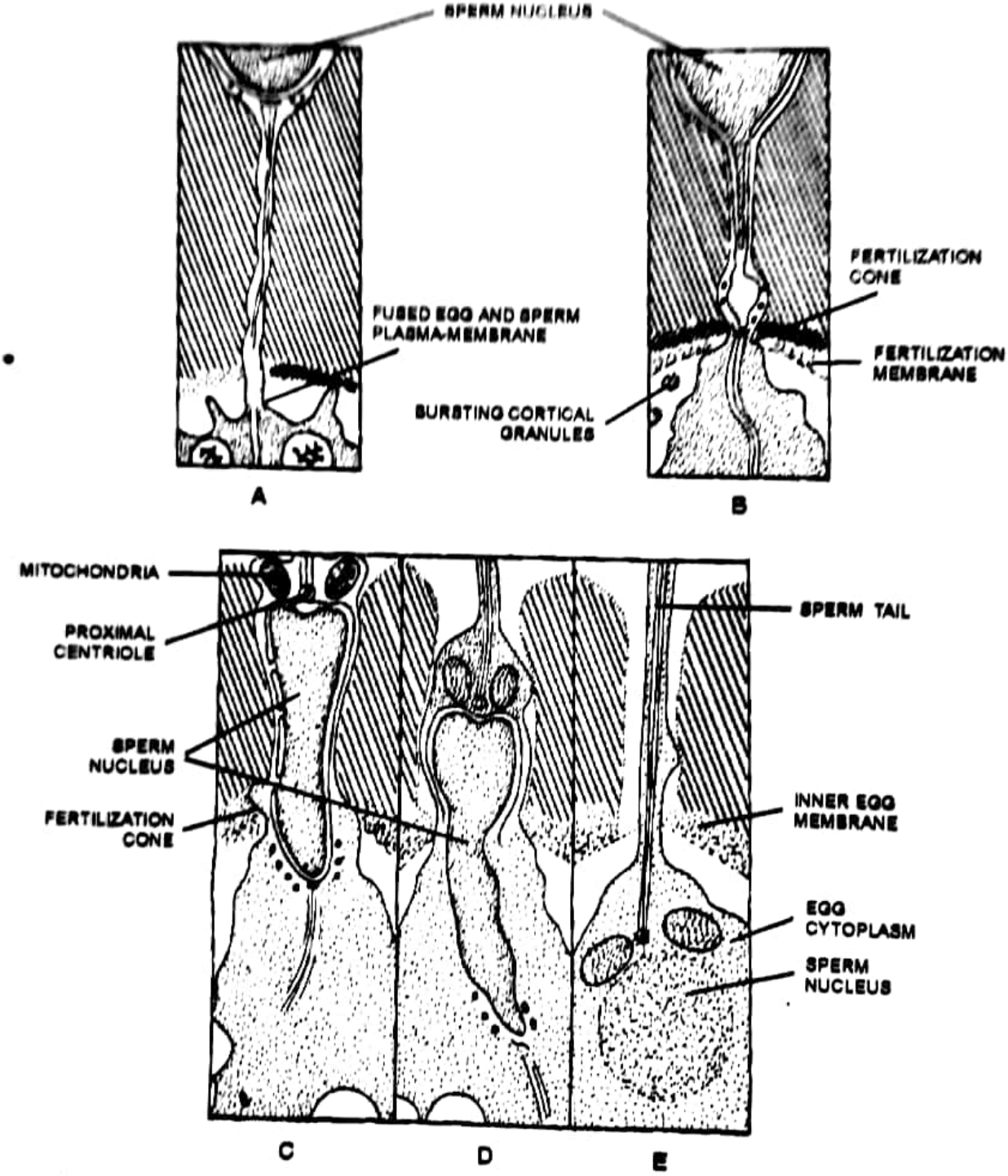

Fig. 4.3. Spermatozoon of *Saccoglossus* penetrating into the egg cytoplasm (after Balinsky, 1976).

the membranes of sperm and acrosome open apically and expose the anterior of the acrosome vesicle to the exterior.

(*ii*) The acrosomal granule is released and comes in contact with the egg envelope. It contains lytic enzymes which make passage through the egg envelopes. Therefore, shortly after its release, the acrosomal granule disintegrates and disappears.

(*iii*) From the shallow depression of the acrosomal membrane, close to the nucleus, a long slender **acrosomal tubule** develops (Fig. 4.2). Soon after, the tubule becomes twice as long as the sperm nucleus.

(*iv*) The acrosomal tubule penetrates through the passage of egg envelope, created by lytic enzymes and fuses with the egg membrane (Fig. 4.3).

(*v*) The fertilization cone protrudes from the egg and engulfs acrosomal tubule. The nucleus of spermatozoon is drawn out towards fertilization cone. The acrosomal tubule dissolves; elongated nucleus along with middle piece of spermatozoon is engulfed into the egg cytoplasm.

Similar events occur in other animals during the penetration of sperm into the egg envelope. But the mammalian spermatozoon though possess acrosomes, do not develop acrosomal filaments. The spermatozoon appears to contact the surface of the egg by its lateral aspect. Following this action, the plasma membranes of the egg and the spermatozoon dissolve at the point of contact, and the spermatozoon is drawn into the interior of the egg.

3. Activation of ovum

Activation of ovum is that aspect of fertilization by which an egg is released from its arrest and begins to develop, as soon as the apex of acrosomal tubule of the spermatozoon touches the surface of the egg plasma membrane. **Berrill** (1971) is of the opinion that periacrosomal material is responsible for the activation of egg. It involves change in ionic permeability of egg plasma membrane (due to activation of various pumps such as proton pump, Na–k pump etc.), transient increase in intra cellular Ca^{++} and pH; the cytoplasmic fusion; fertilization cone formation; cortical reaction and elevation of fertilization membrane; changes in the physical properties and metabolism of the egg; resumption of meiosis and preparation for mitotic cell division for eleavage.

Of all the ions involved calcium seems to play a major role in egg activation. In the absence of calcium fertilization does not occur. Sperm viability, motility and fertilizing capacity are also diminished in calcium free media. Furthermore the acrosome reaction is calcium dependent; without it, no acrosome filament explodes. Egg stability also depends upon calcium; in its absence changes in viscosity and rigidity of the cortex are observed.

A. Cytoplasmic fusion. The plasma membrane of acrosomal tubule fuses with the egg plasma membrane and the two membranes at the region of contact dissolve. Thus, the egg cytoplasm becomes continuous with that of sperm.

B. Fertilization Cone formation. The moment acrosomal filament touches the egg surface, the cytoplasm of the egg bulges forward at the point of contact and produces a process of hyaline cytoplasm called the **fertilization cone.** This cone may be in the form of a conical protrusion or it may consist of several regular pseudopodia like processes (Fig. 4.4) or in some case it may be a cytoplasmic cylinder stretching forward along the acrosomal filament. The fertilization cone gradually engulfs the spermatozoon either completely or a part of it and then begins to retract, carrying the spermatozoon inward. Acrosomal granule never enters the egg, while the periacrosomal material activates the egg.

C. Cortical reactions and the formation of fertilization membrane (Prevention to polyspermy). Sperm and mature egg possess mechanisms that enable them to fuse with each other. A number of molecular mechanisms have been evolved to ensure that sperms fuse only with eggs of the same species at the right stage of maturation. Moreover, normally once an egg is fertilized, it does not fuse with other sperm. It

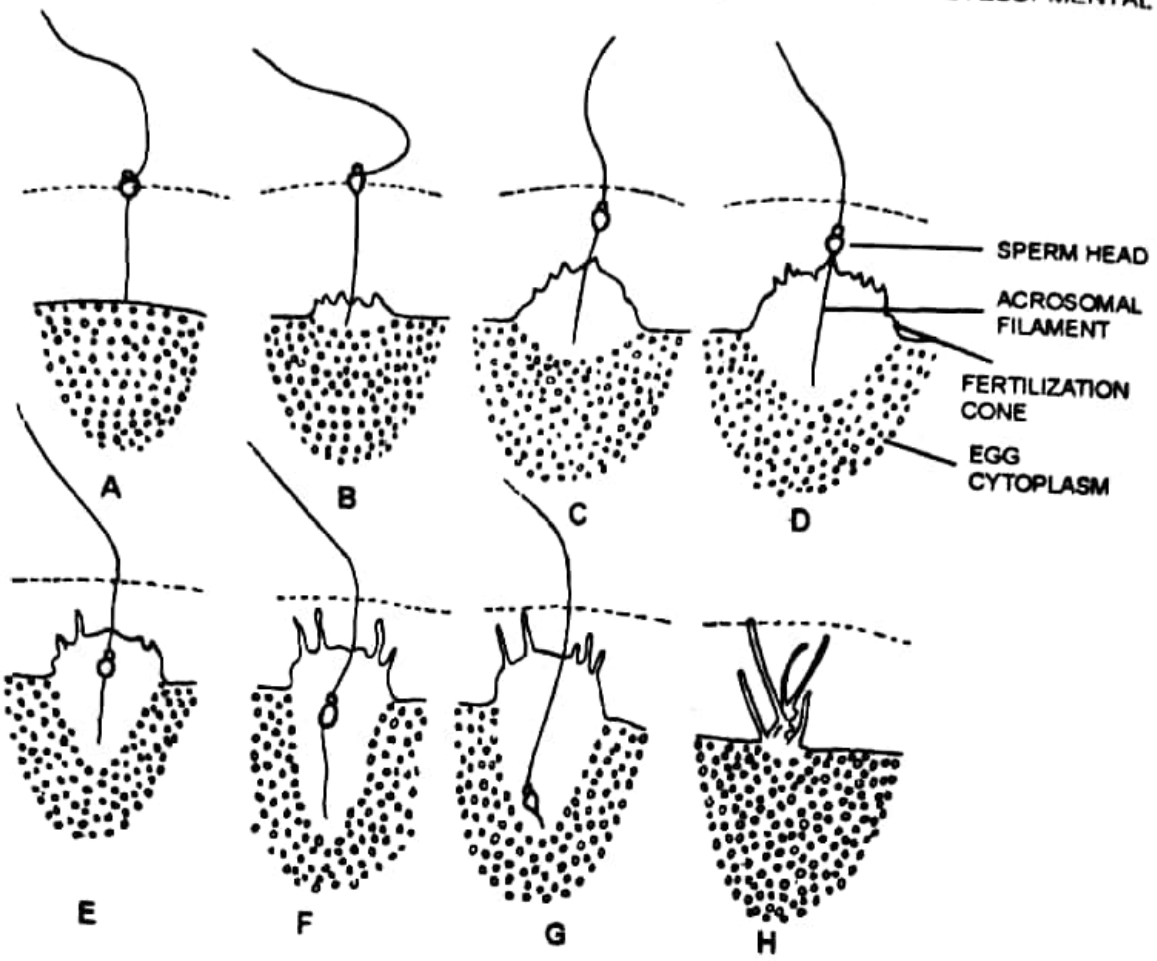

Fig. 4.4. Sperm entry into the egg of sea-cucumber, *Holothuria atra* with the formation of fertilization cone (after Balinsky, 1976).

quickly develops some changes in the plasma membrane preventing the entry of additional sperm. This is vital because in many animals the entry of more than one sperm means entry of supernumerary centrioles and establishment of extra poles for mitotic apparatus causing abnormal cleavage pattern and death of the Zygote.

Entery of the spermatozoon inside the egg results alterations of membrane potential that spread over the egg surface starting from the point of sperm entry (Fig 4.5). *Change in membrane potential causes membrane depolarization involved in the prevention of polyspermy.* An unfertilized egg of sea-urchin has a negative membrane potential of 60 to –80 mv. Within seconds of fertilization the membrane potential reaches a positive value of + 10 to + 20 mv/s. It retains the positive value for 40 to 80 seconds after the initial depolarization. Finally, the membrane potential gradually returns to negative *i.e.*, prefertilization level in about 8 minutes. This changed membrane potential results in altered

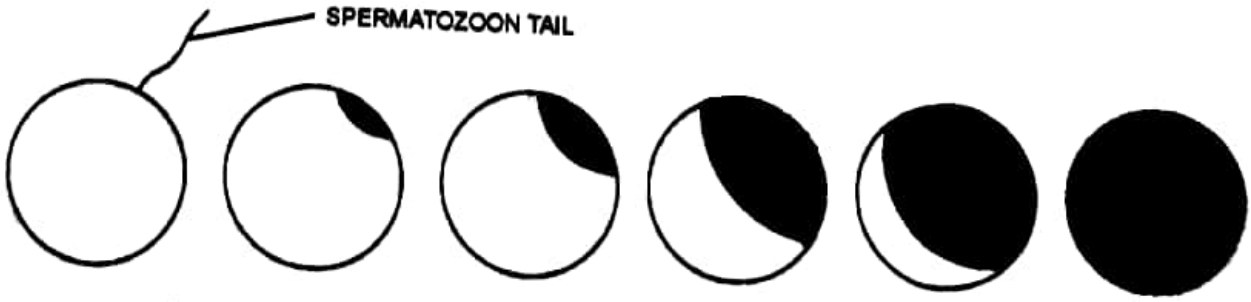

Fig. 4.5. Diagram to show the spreading of alterations of membrane potential (black) in a sea-urchin egg after fertilization (after Balinsky 1976).

membrane permeability to certain ions. Eggs that fail to show depolarization to more positive values become polyspermic. Unfertilized eggs whose membrane potential is experimentally raised, cannot be fertilized. But when current is applied during fertilization to maintain the negative value of resting potential, the eggs become polyspermic. According to **Whitaker** and **Steinhardt** (1983), there is strong evidence to show a correlation between the changed membrane potential and prevention of polyspermy.

In *Xenopus*, a rapid membrane polarization prevents polyspermy. The magnitude of the change in potential and the ions responsible for it may vary from one species to another. In membrane depolarization, it is one of the earliest reactions to egg in binding with the fertilizing spermatozoon in diverse groups of animals. It is reasonable to assume that it constitutes a mechanism of preventing polysermy. It had been investigated thoroughly only in echinoids. However, *membrane depolarization is fast, partial and the temporary block to polyspermy.*

Membrane bound cortical granules located in egg cortex, are products of Oocyte-Golgi complex. Each mouse egg has about 4000 cortical granules while in the eggs of sea-urchin their number exceeds 15,000. As a result of reaction—(*i*) the cortical granules contents are released by exocytosis into the perivitelline space between the egg plasma membrane (60 A° thick) and the vitelline membrane (30 A° thick). In sea-urchin egg the two membranes are closely apposed before fertilization. When the cortical granules are externalized, they release enzymes, structural proteins and mucopolysacchrides. Hydration of mucopolysacchrides is responsible for the elevation of vitelline membrane, now called the *fertilization membrane* which is much thicker upto 900A° and stronger. According to **Louris et. al.,** (1985) sperm *receptor* present on the vitelline membrane are also inactivated by the cortical granule contents. Thus, the cortical reaction that follows the membrane potential alteration acts as permanent physical barrier for the sperm, thereby completely preventing polyspermy (Fig. 4.6).

Another consequence of cortical reaction in sea-urchin egg is—(*a*) egg surface becomes a mosaic of the old and newly surfaced membrane; (*b*) increase in number and size of surface villi; (*c*) doubling of surface area and Con-A binding (**Eddy** and **Shapiro,** (1976); (*d*) burst of retrieval of plasma membrane via uptake of coated vesicles; (*e*) decrease of solubility of surface coat (*f*) **Veron et. al.,** (1977) investigated the morphological changes in the sea-urchin fertilization membrane using scanning electron microscopy and find that as the fertilization membrane elevates from the egg surface it retains dome-shaped (igloo-shape) casts of microvillar tips which soon become angular (tent-shaped).

The fertilization membance, initially permeable to Con-A, now becomes impermeable to it because some molecular cross-linking has introduced stability in the membrane. These changes lead to the hardening of the membrane. **Kay** and **Shapiro** (1987) analysed the isolated fertilization membrane obtained at different intervals following its formation over a period about 20 minutes. The soft membrane solubilized by mercaptan solution and detected that :

(*i*) In membrane extracts obtained during the process of hardening some of the protein components disappeared because they were cross-linked in two distinct ways—(*a*) proteins are bridged by divalent cations; (*b*) Tyrosine residues of the proteins are cross linked by the formation of dityrosine bridges.

(*ii*) The isolated soft membrane can be hardened *in vitro* by the addition of H_2O_2. Under this condition the proteins that disappear from the SDS gels (sodium dodecyl sulphate polyacrylamide gels) are similar to those that disappear during *in vitro* hardening. Thus, it seems that the ovoperoxidase knits huge tyrosine cross-linked macromolecules of MW = 10^{16} D.

In *Xenopus*, the egg plasma membrane is enveloped by vitelline membrane. The jelly coat, a tri-layered tertiary membrane composed of glycoprotein. Following fertilization a sulphated

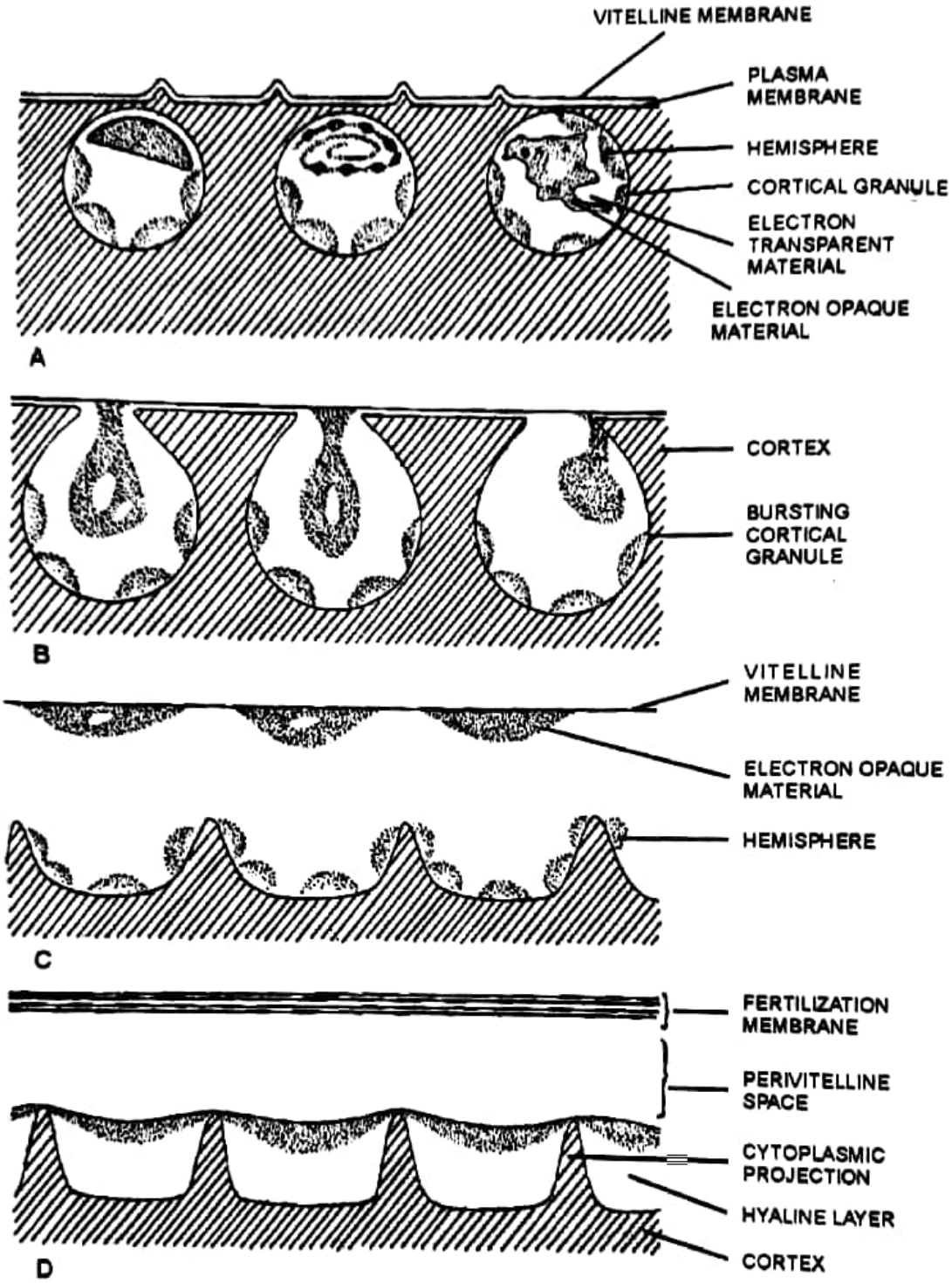

Fig. 4.6 Changes of the egg cortex of the sea-urchin, *Clypeaster Japonicus* following fertilization. A-Unfertilized egg; B-Explosion of the cortical gramules; C-Adhesion of electron opaque material to the vitelline membrane and the formation of fertilization membrane. The hemispheric bodies remain at the surface of the egg and give rise to the hyaline layer; D-The egg surface upon completion of these changes (after Berrill, 1971).

glycoprotein of innermost jelly layer interacts with a component released from the cortical vesicle to form a *'fertilization layer'* that blocks the entry of the additional sperm. The interaction between the glycoprotein and the cortical granules component is akin to lectin-sugar interaction involving α–galactoside residues and Ca^{2+} (**Wolf et.al,** 1976).

(*iii*) In bony fishes and frogs, the cortical granules are broken down immediately after

sperm's penetration into the egg cytoplasm. Their contents become liquefied and extruded on the surface of the plasma membrane of the egg and fill the pervitelline space occurring in between the chorion and plasma membrane in bony fishes and in between the vitelline membrane and egg plasma membrane in the frog. The chorion in fishes become hardened or **"tanned"** after fertilization and no fertilization membrane is formed in either of the two animals.

In some mammals (man, rabbit etc.,) the cortical granules burst and release their contents into the perivitelline space created between the egg plasmalemma and the zona pellucida.

Urodele amphibians and some mammals (rat and guinea pig) lack cortical granules, therefore, no cortical reaction occurs. However, in caudate amphibians a perivitelline space is formed in fertilized egg (**Lovtrup,** 1974).

From the literature reviewed above it may be concluded that there are three mechanisms to prevent polyspermy. (*i*) A fast block to polyspermy is due to membrane depolarization within seconds of fertilization. (*ii*) An intermediate block starts about 30 seconds after sperm addition and lasts till hatching due to (*a*) a steric effect because the cortical reaction elevates a fertilization membrane which prevents sperm to reach the egg surface; and (*b*) a trypsin like protease released from the cortical granules, degrades binding receptors from vitelline membrane and detaches the bound sperm. (*iii*) A late block between 10 and 15 minutes after fertilization is dependent on the discharge of cortical granules and appears to be permanent. This block is effective only against modest sperm concentration. The three mechanisms together are very effective block to polyspermy. In sea-urchin the sperms also reduce their fertility in response to electrical and chemical signals produced by the fertilized egg.

4. The essence of activation

After the penetration of spermatozoon in an unfertilized egg, a series of rapid changes occur—(*a*) the reduction division, if not performed before, is brought to completion; (*b*) the male and female pronuclei fuse; (*c*) dislocations of cytoplasmic substances of the egg may take place; and (*d*) the egg enters the period of cleavage by rapid mitotic cell divisions. Following **metabolic changes** also occur in the egg at fertilization :

(*i*) Changes in plasma membrane. The permeability of plasma membrane increases for the molecules of water, ethylene, glycol and phosphate. The potentiality of the membrane is more positive in the beginning but gradually becomes more negative. Beside this a plasma membrane enzyme (adenylcylase) becomes activated at the time of fertilization, and it starts the formation of a chemical molecule 3′ – 5′ – cyclic **AMP,** which activates most of the metabolic reactions in a fertilized egg. (**Castaneda** and **Tyler,** 1968).

(*ii*) Coenzyme changes-NAD-kinase enzymes exists in the unfertilized egg in an inactivated stage. It is made activated only at the time of fertilization (**Epel** and **Iverson,** 1965).

In a fertilized egg, there occurs an inter-conversion of pyridine nucleotide coenzyme, NAD into another coenzymes NADP and also NADPH due to the phosphorylation of NAD in the presence of a NADkinase.

$$NAD + ATP \xrightarrow{NADkinase} NADP + ADP$$

Since the coenzymes NAD and NADP are involved in numerous enzyme mediated oxidation and reduction reactions of the cell, any change in the concentration or availability of these enzymes may have profound metabolic consequence.

(*iii*) Ionic changes. Certain intracellular changes occur in the concentration of ions, especially those of sodium, potassium and calcium in fertilized egg (**Rasmussen,** 1970).

(*iv*) Changes in the rate of respiration. After fertilization there is sharp increase in oxygen consumption indicating an increase in the rate of respiration. The spermatozoon activates the oxidative enzymes of the egg that provides energy necessary for the performance of the other

changes in the egg and for the development of the egg in general. Moreover, there appears some relation between the post-fertilization oxygen consumption and the stage of maturation of egg at fertilization. In sea-urchin egg, which has completed maturation divisions at the time of fertilization, the rate of respiration increases; in the egg of *Chaetopterus* which is at the first maturation stage, the rate of respiration decreases; while in the egg of *Bufo* and *Fundulus* which is at the second maturation division, the rate of respiration remains static.

(*v*) Changes in the rate of protein synthesis. The cytoplasm of a mature unfertilized egg, though contains complete machinery for protein synthesis (such as DNA molecules, *m*RNA, ribosomes and proteolytic enzyme), none or very little protein synthesis occurs because the *m*RNA of unfertilized egg remains **"masked".** **Metafora** *et. al.*, (1971) have indicated that during later phase of oogenesis in sea-urchin egg, some inhibitor or repressor proteins are manufactured which inactivate chromosomal genes, *m*RNA molecules, ribosomes etc. During fertilization some proteolytic enzymes remove these inhibitors from them and unmasks the *m*RNA and activate protein synthesis. In the egg of frog, however, the increase in the protein synthesis starts quite early at ovulation itself (**Smith** *el. al.* 1966).

(*vi*) Initiation of mitosis. For the initiation of mitotis for cleavage—(*a*) the rate of DNA synthesis increase with great pace immediately after fertilization due to selective accumulation or uptake of cytoplasmic **DNA-polymerase** enzyme (an enzyme used in DNA synthesis) by the nucleus (**Loeb** and **Fansler,** 1970) (*b*) the unfertilized egg cytoplasm though has a centriole, yet this centriole incapable to form a mitotic spindle.

The sperm stimulates for the first mitotic division (cleavage) of fertilized egg by contributing its cenriole to the egg.

5. Theories of activation of eggs

(*i*) Boveri's Theory. Boveri (1887 and 1895) suggested that the egg loses its power of mitotic division during maturation as its division centre degenerates. The sperm introduces the division centre into the egg during fertilization and makes the egg to divide.

(*ii*) Loeb's Theory. Loeb (1964) believed that in normal fertilization the sperms bring in a lytic principle which brings about cortical cytolysis and the "corrective factor" which regulates oxidation. **Lovtrup** (1974) has supported the Loeb's theory and suggested that lytic substances function as activators by causing a partial hydrolysis and perforation of plasma membrane, dissolution of cortical granules and subsequent swelling of pervitelline space.

(*iii*) Bimolecular Theory. Lillie (1941) postulated that the cortical changes are the main aspects of activation of egg. The decrease in viscosity permits the interaction of various cytoplasmic substances which normally are kept separated in the unactivated egg. The activating substance 'X' formed in the egg, is comparable to the growth of hormone. This substance is formed by the union of two substances 'Y' and 'Z' that may be initially present in low concentration in the egg. Substance 'Y' is considered to be a product of hydrolytic processes in the egg and its formation to be stimulated by the action of such agents as acids and heat, which can act under anaerobic conditions. Substance 'Z' is considered to be formed by synthetic processes that may be stimulated by agents like hypertonicity acting only in aerobic conditions. Since some substance of each type is present, the threshold concentration of 'X' can be reached by an increase in either 'Y' or 'Z'.

(*iv*) Bataillon's theory. Bataillon (1910,' 11,' 13 and ' 16) placed emphasis on the exudation or the excretion of substances into perivitelline space and elevation of the fertilization membrane. He believed that the unfertilized egg was inhibited because of an accumulation of metabolic products and the activation or fertilization led to release of these substances to the exterior of egg.

(*v*) Sensitization to calcium theory. The importance of calcium in egg activation has been emphasized in two theories–"*Sensitization to*

calcium" theory of **Dlacq, Pasteels** and **Brachet** (1936) and the "*calcium release protoplasmic clotting*" theory of **Heilbrunn** (1930–'40). These theories revealed that—(*a*) isotonic calcium chloride solutions or calcium rich solutions are effective activators in many species of animals; (*b*) activation of other chemical agents is dependent upon the presence of calcium ions; and (*c*) depriving the eggs of calcium will sensitize them to the subsequent action of calcium solution. Artificial parthenogenetic studies show a significant role of calcium in the activation of egg.

(*vi*) **Repressor theory. Monroy** (1965) has given convincing arguments about the activation of egg by sperm in molecular terms. According to him, during the later phases of maturation, some repressor substances are manufactured by the egg which inhibit both the energy yielding metabolic activaties residing in the cytoplasm and the genetic activities of the nucleus. Monroy's repressor has been supported by many modern embryologists, such as **Metafora** *et. al.*, (1971), **Berrill** (1971) and **D. Epel** (1973) etc. The key reaction of fertilization is thus, the removal of the repressors, releasing at the same time the cytoplasmic metabolic activities and the activity of the nuclear genetic system. However, how such inhibitions are produced during maturation and how they are removed following fertilization still remains to be discovered.

6. The spermatozoa in the egg interior, migration of pronuclei and the site of amphimixis.

Variations have been observed in different groups of animals, as to how much of the spermatozoon is engulfed into the interior of egg, during fertilization process. In most cases, the sperm periacrosomal material, nucleus, proximal centriole and mitochondria make their entry as a rule. The plasma membrane of sperms becomes one of the entity of plasma membrane of the egg. But acrosomal granule never make its entry into the egg. In mammals, complete structures of spermatozoon (*i.e.*, head, middle piece and tail) penetrate into the egg cytoplasm. In echinoderms the sperm tail remains exterior to the vitelline membrane while in Nereis, only the sperm nucleus and proximal centriole enter the egg cytoplasm. But only the nucleus and centrosome play an active part in subsequent development.

Migration of pronuclei. The spermatozoon in the egg cytoplasm moves with acrosome or acrosomal filament at its front and nucleus, centrosome and mitochondria are carried behind it. As the sperm nucleus moves inward from the site of fertilization cone, to the site of amphimixis, it rotates an angle of 180° so that its mitochondria and centrosome assume the leading position. Besides, the chromatin of sperm, nucleus becomes finely granular and the nucleus itself becomes vesicular by absorbing fluid from the surrounding cytoplasm. It is now called **male pronucleus.** At the same time, the sperm aster forms in the cytoplasm around the proximal centriole of the sperm. As the male pronucleus migrates toward the site of amphimixis, the sperm aster leads it. As the sperm pronucleus along with the centriole moves inward, it may be accompanied by some

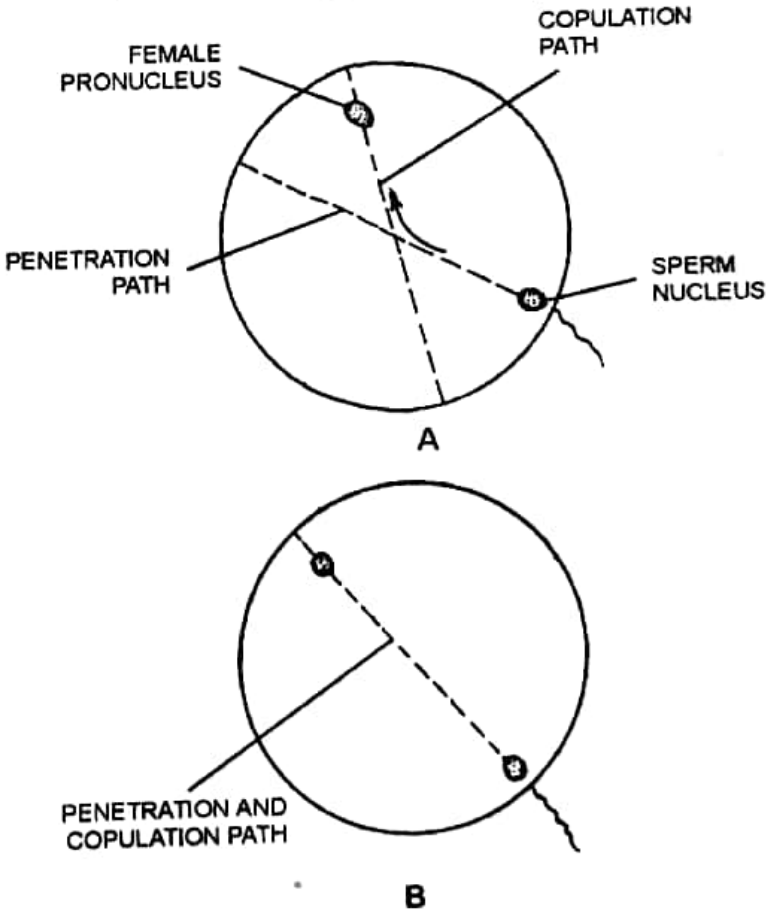

Fig. 4.7. Possible sperm paths during fertilization (after Manner, 1964).

cortical and sub-cortical cytoplasm. If the latter is heavily pigmented, as in amphibian eggs, the path of sperm pronucleus may be marked by pigmented granules trailing along its path. This is called **penetration path.** This movement of the sperm appears to be directed, and some investigators feel that it is due to a chaemotaxic effects of chemicals liberated by the female pronucleus. During this movement toward the female pronucleus, the sperm may have to deviate from its penetration path. If it does, the new pathway is taken. This is referred to as the **copulation path** (Fig. 4.7).

In some cases the sperm need not alter its direction. In these cases the penetration and copulation paths would be identical. The point of entrance, penetration path and copulation path have all been postulated responsible for establishing the primary plane of **bilateral symmetry** in the embryo.

The nucleus of the egg also undergoes certain changes like the sperm nucleus. By the end of second meiotic division, the haploid egg nucleus lies in the peripheral cytoplasm in the form of several vesicles known as **karyomeres.** These karyomeres fuse with each other to form the **female pronucleus.** It swells and increases in volume as it approaches the male pronucleus. In the last stage before they meet, the male and female pronuclei may become indistinguishable (**Balinsky,** 1976).

Amphimixis. The fusion of male and female pronuclei is called the **amphimixis.** The site of amphimixis lies near the centre in microlecithal eggs, while in mesolecital and macrolecithal eggs it lies in the centre of active cytoplasm at the animal pole. The actual fusion of pronuclei may differ in different animals.

(*a*) In sea-urchin and vertebrates, the nuclear membranes of both pronuclei are broken down at the point of contact and the contents unite in one mass surrounded by a common nuclear

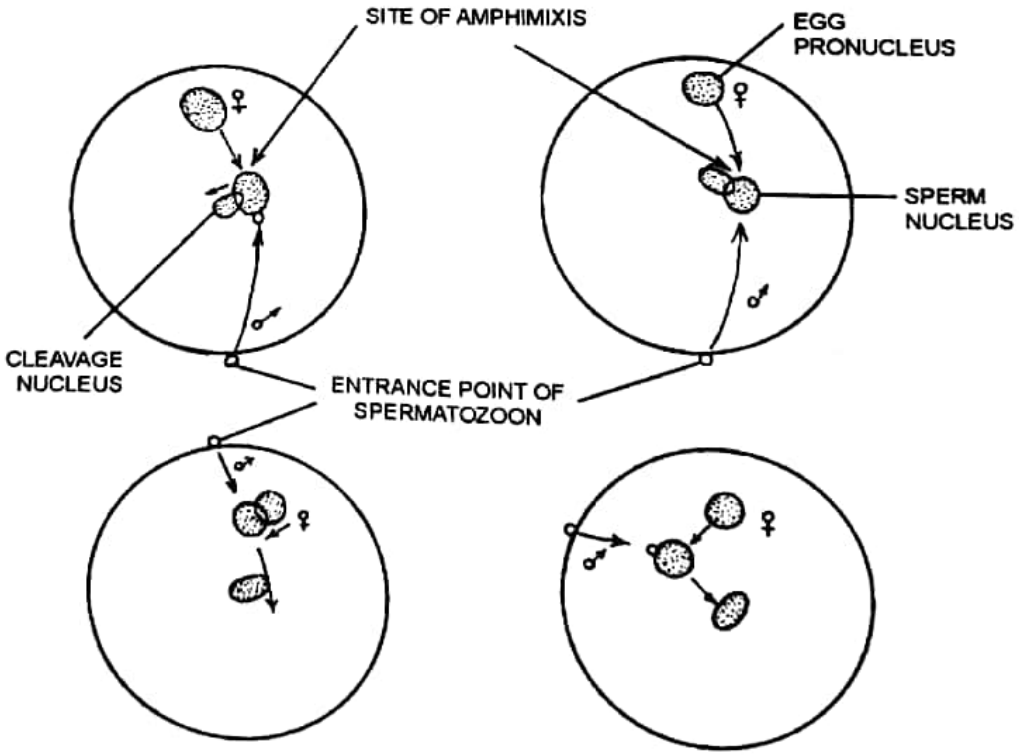

Fig. 4.8. Amphimixis in sea-urchin. Tracks of the pronuclei in their movements toward each other and to the site of the cleavage nucleus, with sperm entering at opposite side of the egg from the site of egg nucleus and from the same side, respectively (after Balinsky, 1970).

membrane to form **Zygote nucleus** (Fig. 4.8). At the approach of first cleavage of fertilized egg, the nuclear membrane dissolves, chromosomes of maternal and paternal origin become arranged on the equator of the achromatic spindle.

(*b*) In *Ascaris*, some molluscs and annelids, the male and female pronuclei do not fuse but the nuclear membranes in both dissolve and the chromosomes become arranged on the equator of the spindle. In these animals the **zygote nucleus** is not formed. Only after the completion of the first division of the fertilized egg, the paternal and maternal chromosomes become enclosed by common nuclear membrane to form the nuclei of two blastomeres into which the egg has been divided.

(*c*) In *Cyclops*, the paternal and maternal nuclear components remain separate for some time even after cleavage has started, so that each blastomere has a double nucleus consisting of two parts lying side by side and each surrounded by its own nuclear membrane.

Changes in the organization of the egg cytoplasm caused by fertilization (post-fertilization changes).

In addition to the activation of the egg cytoplasm and the restoration of diploid chromosome number, the penetration of spermatozoon into the egg causes reorganization of egg cytoplasm and also changes the symmetry of the egg. The distribution of various cytoplasmic substances and inclusions in the fertilized egg may be very considerably different from that in the unfertilized egg, and even qualitatively new areas may appear. Due to the extrusion of cortical granules, the original outer egg cell surface is replaced by the inner surfaces which surrounded the cortical granules and now are everted on to the exterior. Most spectacular post-fertilization displacements in the egg cytoplasm has been observed in ascidian, *Styela partita* and in frog. In both these animals, there establishes a bilateral symmetry in the cytoplasm of fertilized eggs.

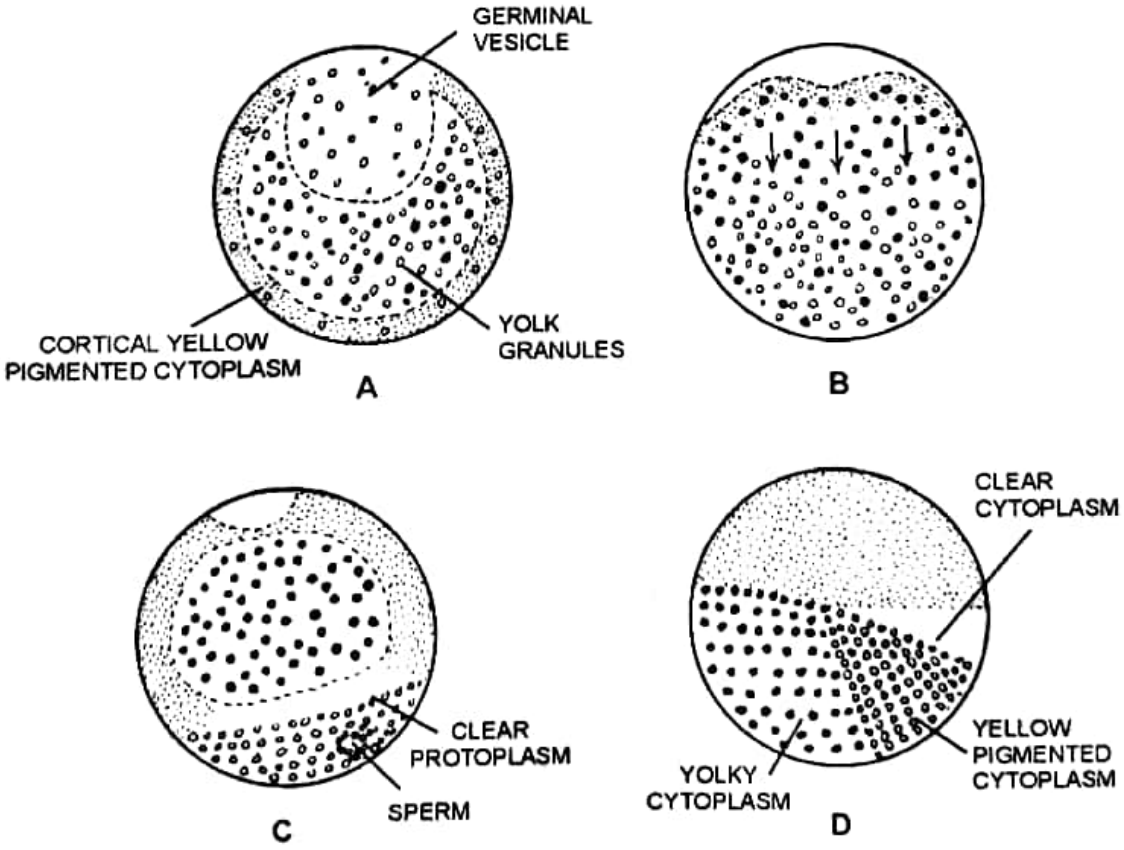

Fig. 4.9. Displacements of cytoplasmic substances in the egg of the ascidian, *Styela partita* following fertilization. A – Ripe unfertilized egg; B – Egg soon after the entry of spermatozoon; C – D – Male pronucleus moves to meet female pronucleus and the formation of cytoplasmic territories (after Balinsky, 1976).

In *Styela partita*, (Fig. 4.9), the moment spermatozoon enters the egg near the vegetal pole, the yellow cortical cytoplasm begins to stream down along the surface of the egg towards the vegetal pole, and accumulated as a cap. As the male pronucleus penetrates deeper into the cytoplasm and moves toward female pronucleus, the yellow cytoplasm reverses its movements and streams upward only on the side where the spermatozoon extend the egg. Just before the first cleavage the yellow cytoplasm forms a crescentic area (mesodermal crescent) just below the equator of the egg. Simultaneously, the crescent of light gray cytoplasm (notochordal crescent) appears subequatorially on the opposite side of the egg. These two together form a belt surrounding the egg just below the equator. Below this zone toward the vegetal pole, the cytoplasm is slaty gray in colour and contains abundant yolk granules and in the subcortical layer, there are large number of mitochondria. But the cytoplasm in the animal hemisphere contains less yolk and few mitochondria and appears more transparent. Thus, the cytoplasmic displacements following fertilization not only bring some kind of cytoplasm to more restricted areas, but also give the egg a distinct bilateral structure (**Colwin,** 1905).

Significance of fertilization

(*i*) Gametes constitute the only physical bridge between the successive generations. Amphimixis restores the diploid chromosomal configuration similar to parents.

(*ii*) Fertilization brings together the genes and chromosomes from two lines of ancestry and results in new genetic recombinations, which ensures better adaptations to the changing environments.

(*iii*) Liberation of secondary oocyte to complete its second maturation division; induces movement of cytoplasmic particles and pigment granules to mark the fertilization track.

(*iv*) Induces centriole which forms the nuclear spindle and initiates cleavage in the egg ; formation of fertilization membrane precludes entery of other sperms.

(*v*) Causes separation of vitelline membrane which allows rotation of egg inside.

(*vi*) The entry of sperm awakens the quiescent ovum and initiates the egg to divide and proceed the development.

(*vii*) Enhances the metabolic activities and establishes a new plane of egg axis.

Time of fertilization

It has been found that spermatozoon enters the ovum at different stages of maturation in different animal species. Thus, the time of fertilization varies from species to species.

In *Ascaris, Grantia, Myzostoma, Nereis,* dog, fox etc., the spermatozoon enter the primary oocyte.

In *Cerebratulus, Chaetopterus, Dentalium,* many insects and ascidians the egg is being fertilized at metaphase of the first meiotic division stage; while an *Amphioxus* and most vertebrates at metaphase of second meiotic division.

Types of fertilization

Normally only one spermatozoon penetrates into the egg in most classes of animal kingdom (*e.g.*, coelenterates, echinoderms, bonyfishes, frogs and mammals) to fertilize the egg is called **monospermic fertilization.** But under certain pathological conditions many spermatozoa penetrate the ova of a normally monospermic animal, such a type of fertilization is called **pathological polyspermy.** However, the ovum fails to develop further and dies soon. There are certain groups of animals mainly those having yolky eggs, such as some molluscs, selachians, urodeles, reptiles and birds; many spermatozoa may penetrate ova as a whole. Such kind of fertilization is called **polyspermic fertilization.** In such eggs only one spermatozoon participates fully in the development of the embryo, the rest degenerate sooner or later. This type of polyspermy, because, has some physiological significance, therefore, is called **physiological polyspermy.**

In *Rhabditis aberrous* where the ovum is activated by the entry of sperm but its pronucleus fails to fuse to form zygote, is called **Gynogenesis.** But when the egg is activated by

the sperm and development takes place without the participation of the egg nucleus, the type of fertilization is **Androgenesis.** Thus, in eggs developing by androgenesis, the nuclei of the embryo are of paternal origin only.

In **polygamy** the two female pronuclei (female pronucleus and polar body) fuse with the single male pronucleus as in urodeles and rabbits. **Polyandric** condition has been reported in man where two male pronuclei (sperms) unite with female pronucleus.

In **Merogamy** egg fragments devoid of the nucleus develop when fertilized by a normal sperm *e.g.*, Sea-urchin. In **partial fertilization** the egg may be fertilized by a part of the sperm. **Boveri** has reported the fertilization of the egg by the sperm aster in sea-urchin.

Chemistry of fertilization

Both egg and sperm contain certain chemicals that are necessary for fertilization. Since, these chemicals are produced by the gametes and are of the nature of hormones, are known as **gamones.** The hormones of the sperm called **androgamones**. These are of two types– **androgamone I** and **androgamone II**. The hormone *androgamone I* is responsible for conserving sperm's activity. Since the sperm contains limited amount of energy and if it were to swim as rapidly as it could from the very beginning its limited energy would be utilized in too short period and sperm may not be able to reach the ovum. By conserving the activity until the sperm approaches the egg, the probability of fertilization is increased. **Androgamone** dissolves the gelatinous coating around the egg and is, therefore, allowing **sperm enterance** into the egg.

Hormones those found in egg are called **gynogamones** and are also of two types. **Gynogamone I** produced in the ovum neutralizes the androgamone I when sperm comes in contact with the egg and thereby **increases the activity of the sperm. Gynogamone II** makes the sperm head stick and **enables the sperm to strick** to the egg surface and allows the androgamone II an opportunity to dissolve gelatinous coating.

REVISION QUESTIONS

...genesis growth period.

(b) Vitellogenesis growth period—The vitellogenesis growth period is the period of rapid growth, because, during this period the egg cytoplasm is packaged by reserve food materials, such as, glycogen, certain other carbohydrates, lipids and proteins, all of which collectively called **yolk** or **vitellin.**

PHYSICO-CHEMICAL NATURE OF YOLK

Yolk is the usual form of food storage in the growing oocyte and egg. Chemically, it is composed of proteins, phospholipids and neutral fats. It may be called "**protein yolk**", when it has more proteins than lipids, or "**fatty yolk**", when it has more fat contents than the proteins. Most animal eggs contain both kinds of yolks. Both of these kinds of yolk occur in following two forms :

1. Granular yolk—In the egg of invertebrates and lower chordates such as *Amphioxus* and tunicates, the protein yolk is found

in the form of fine granules which remain evenly distributed in the cytoplasm of oocyte.

2. Yolk platelets—In case of most vertebrate groups, namely, cyclostomes, elasmobranchs, ganoids, lung-fishes, amphibians, reptiles and birds, the yolk occurs in the form of large granules, called **yolk platelets**. The yolk platelets of amphibians, for example, have an oval shape and are flattened in one plane. The cytoplasm is densely packed with them. Chemically, the yolk platelets of amphibian oocytes contain two main proteinaceous substances—**phosvitin and lipovitellin**. Phosvitin is a highly phosphorylated protein (phosphorus content, 8·4 per cent), with a molecular weight of 35,000. The lipovitellin is a protein with a very much larger molecule (molecular weight 40,000) and containing a very considerable amount of bound lipid (17·5 per cent). In the yolk platelets two molecules of phosvitin are associated with one molecule of lipovitellin in a structural unit. The units are being arranged in the platelet in a crystalline lattice with regular hexagonal packing. Other animals have yolk platelets similar to amphibians, however, the yolk of some bony fishes has fat droplets inside its mass.

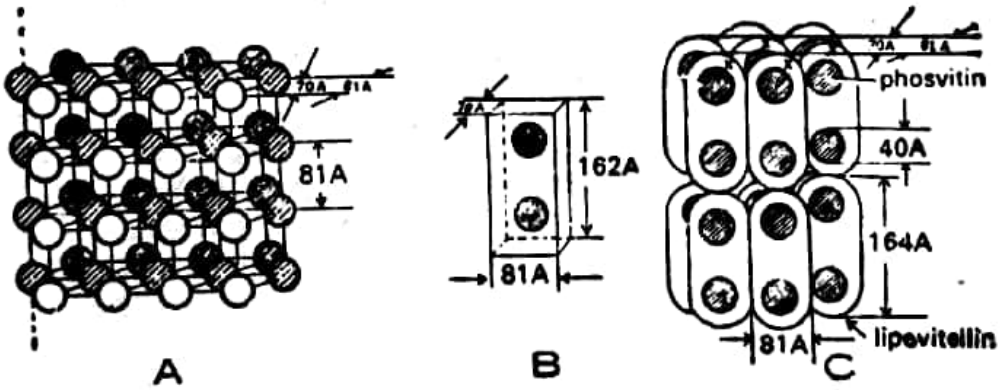

Fig. 3·7. Diagram of crystalline structure in the main body of an amphibian yolk platelet. A—Arrangement of phosvitin molecules in the lattice; B—A structural unit consisting of two molecules of phosvitin and one molecule of lipovitellin; C—Arrangement of phosvitin and lipovitellin molecules in a simple hexagonal lattice (After Balinsky, 1970).

YOLK FORMATION OR VITELLOGENESIS

Although in the some species, the yolk may possibly be synthesized by the oocytes themselves, but, according to most recent embryological investigations, the **vitellogenesis** or synthesis of different yolk components (such as proteins and phospholipids) occurs in some extraovarian tissue, such as fat bodies in insects and liver in most vertebrates. From the liver, the yolk components are transported in **soluble state** to some ovarian cells, such as **follicle cells**, by the blood stream. As a developing oocyte of vertebrate remains

surrounded by follicle cells, so, from these follicle cells, the oocyte takes them (*viz.*, yolk components) through their microvilli by the

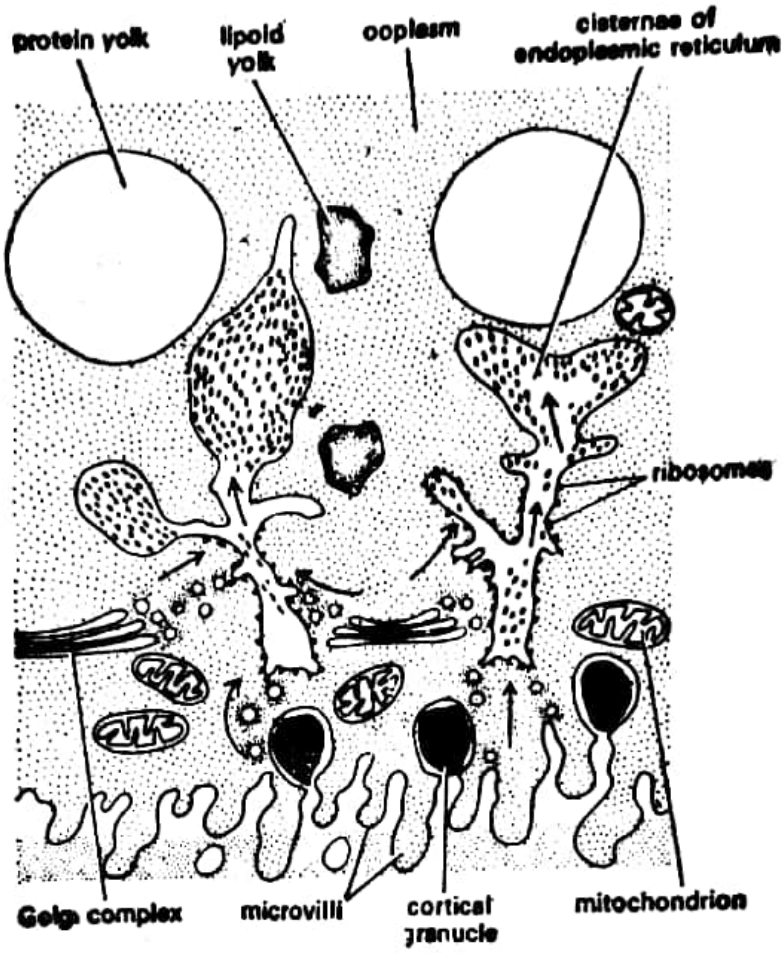

Fig. 3·8. Vitellogenesis in an insect. The lipid component of yolk first accumulates into follicle cells and then, is transferred from them to the oocyte. The protein component of yolk is either manufactured in mid gut and then transferred to ovaries by blood or synthesized in follicle cells. It is either taken directly from the haemocoel or indirectly through the follicle cells by the oocyte, by the act of pinocytosis. Pinocytic vesicles from the periphery fuse with the endoplasmic reticulum and these are joined by vesicles from the Golgi complex. Materials accumulate in the cisternae of endoplasmic reticulum and eventually form the protein yolk spheres (After Berrill, 1971).

process of pinocytosis. The Golgi complex and endoplasmic reticulum transport these yolk components to the modified mitochondria of oocyte (Fig. 3·9). Inside the mitochondria, these yolk components are made insoluble by a mitochondrial enzyme called **protein kinase** and ultimately, **yolk granules** or **yolk platelets** are synthesized. During the crystallization process of yolk platelets, the mitochondrial cristae become dislodged and their membranes ultimately become arranged in concentric layers while, the whole mitochondrial space is taken up by the main body of yolk platelet.

AMOUNT OF YOLK AND TYPE OF EGGS

The amount of accumulated yolk contents of growing oocyte

which is destined to become mature, fertilizable egg after maturation division, varies from species to species of chordates. According to the amount of yolk following kinds of chordate eggs have been recognised:

(1) Microlecithal eggs—The ova or eggs containing small amount of yolk and other reserve food substances are called **microlecithal ova or eggs** (**Torrey**, 1971). Some embryologists have described microlecithal **eggs as alecithal** eggs (**Kent** 1969) or **oligolecithal** eggs (**Romer**, 1962 and **Ballnsky**, 1970) **Example** : *Amphioxus* and other cephalochordates, tunicates and eutherian mammals.

(2) Mesolecithal eggs—The ova or eggs containing moderate amount of yolk are called **mesolecithal eggs. Example** : Petromyzontia, Dipoin, and Amphibia.

(3) Macrolecithal or polylecithal eggs—The macrolecithal or polylecithal eggs contain enormous food reserves or yolk. **Example** : Myxinoidea ; Chondrichthyes, Osteichthyes, reptiles, birds and Monotremata.

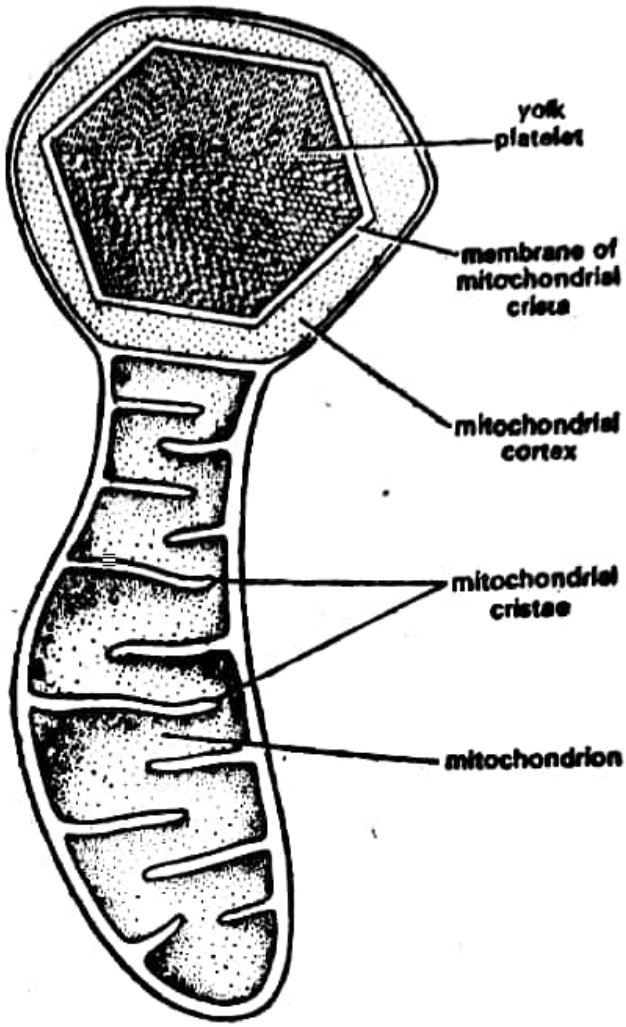

Fig. 3·9. Formation of yolk crystal inside a mitohondrion of a frog oocyte (After Balinsky, 1970).

In microlecithal eggs, the amount of yolk is so little that it remains scattered evenly throughout the egg cytoplasm. Such eggs with evenly distributed yolk are called **homolecithal** or **isolecithal eggs**. In **mesolecithal** and **macrolecithal** eggs, the yolk, due to its gravity, is concentrated more in one hemisphere than in the other, and such kind of eggs are called **telolecithal** eggs. Because of the uneven distribution of yolk, such an egg is said to have a **vegetal pole**, where the concentration of yolk is greatest and an **animal pole**, where the concentration of yolk is smallest. In fact, in macrolecithal eggs, the amount of yolk is so massive that it almost occupies most of the space of animal pole, and the active cytoplasm and germinal vesicle (nucleus) remain confined to a small cap at the animal pole.

In insect eggs, the yolk is concentrated in the centre of eggs and their eggs are called **centrolecithal eggs.**

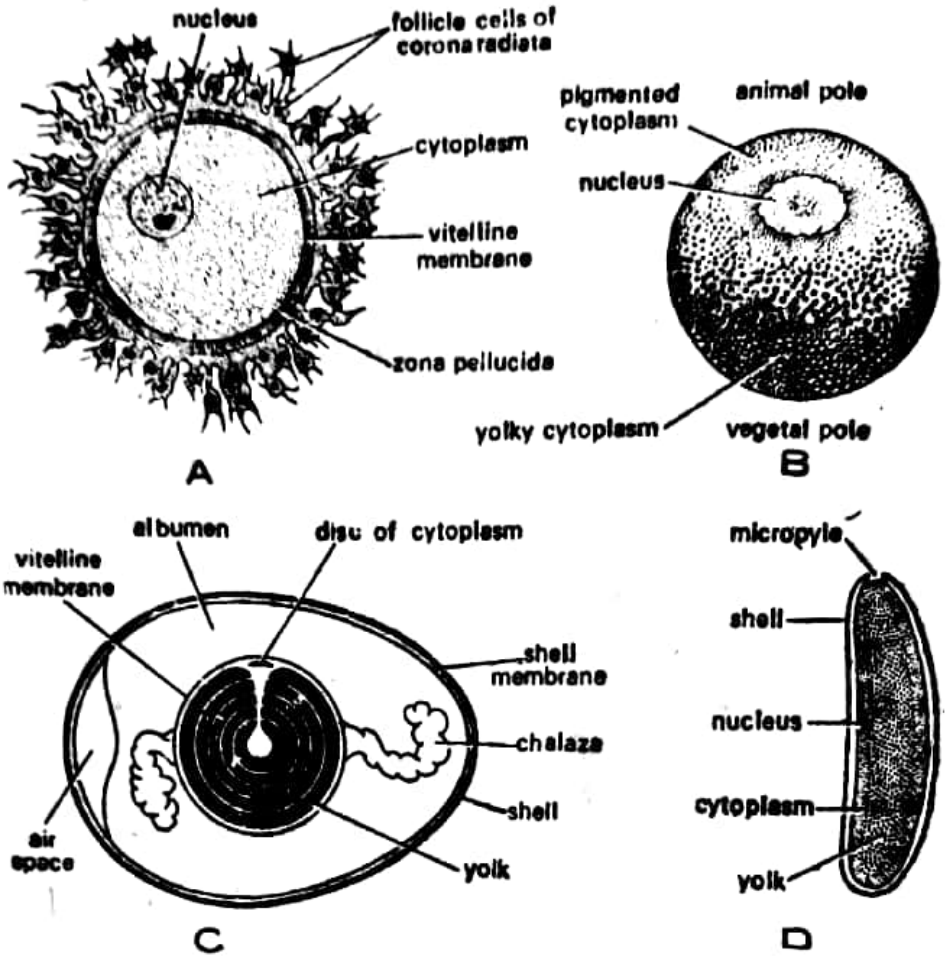

Fig. 3·10. Representative types of ova. A—Microlecithal and isolecithal ovum of man; B—Mesolecithal and moderately telolecithal ovum of the frog; C—Macrolecithal and highly telolecithal egg of the hen; D—Macrolecithal and centrolecithal egg of an insect, the fly (After Arey, 1965 ; and Balinsky 1970).

Thus, there exists a great variation in different animal groups with regards to the accumulation of nutritive reserves, the yolk, inside ooplasm. It is interesting to note that phylogenetic evolution in the vertebrates has been accompanied by a gradual increase in reserves, reaching a maximum in Reptilia and Aves, followed by a drastic reduction in Mammalia (see **Soren Lovtrup**, 1974).

FUNCTIONS OF YOLK

The reserve material (*i.e.*, yolk, lipid droplets and glycogen granules) of egg is used for two purposes—supply of energy and synthesis of the products required for elaboration of the embryonic body. The yolk also exercises an important influence on the size of egg, on differentiation of ooplasm, on patterns of cleavages, on the orderly movements (**morphogenetic movements**) of blastomeres during gastrulation and on the type of development (whether indirect with

larval forms or direct with juvenile stages). The fully grown diploid primary oocyte, now, resembles with the haploid egg, therefore, most embryologists have normally described it as a unfertilized egg.

FUNCTION OF OVARIAN TISSUES DURING GROWTH OF OOCYTE

In chordates, the oocytes are surrounded during whole time of their growth and maturation by special cells of the ovary, the **follicle cells**. In mammals the follicle cells are derived from the germinal epithelium of the ovaries, and initially the young oocyte is surrounded by one layer of follicle cells, forming a simple cuboidal epithelium around the oocyte. Later, the number of follicle cells increases greatly, and cells become arranged in several layers. As the oocyte approaches maturity, an eccentric, fluid-filled cavity called **antrum** appears in the mass of the follicle cells. The follicle at this stage is called **Graafian follicle.**

Electron microscopical studies have shown that the plasma membranes of follicle cells and of an early oocyte remain separated by a narrow gap of 80 Å. At a later stage a wider space appears between the follicle cells and the oocyte. The follicle cells, however, maintain their close contacts with the developing oocyte, by the help of **desmosomes** which occur between the plasma membrane of both types of cells. The cytoplasm of follicle cells becomes drawn out into elongated processes or **microvilli**, which reach across the space separating the follicle cells and the oocyte. At this time, the surface of the oocyte is drawn out into numerous finger-like microvilli projecting into the space between the oocyte and follicle cells. Ultimately the microvilli of the occyte interdigitate with those of the follicle cells. This zone of microvilli in mammals is called **zona radiata**. The presence of the microvilli greatly increases the surface area of oocyte. These are the areas which facilitates metabolic turn over between oocyte and its environment (follicle cells).

At the base of microvilli of oocyte, some inpocketings of the oocyte cytoplasm, called **pinocytes** are formed. Oocyte takes in necessary chemical molecules from the space between oocyte and follicle cells by means of pinocytosis. It is found that the follicle cells actively assist the growth of the oocyte by secreting various chemical macromolecules, which are ultimately taken up by the oocyte.

Nurse cells—In some insects, molluscs and annelids, the relationship between the oocyte and rest of the ovary is further complicated by the presence of special **nurse cells** which, together with the follicle cells, take part in providing the nutrition to growing oocytes. Nurse cells are derived from the primordial germinal cells during multiplication phase and later on, instead of becoming the oocyte.

become the **nurse cells**. No microvilli are developed at the interface between the oocyte and nurse cells. The nurse cells either become used up during the growth of oocyte (in some insects and annelids) or they may be completely engulfed in the cytoplasm of the oocyte (in the snail, *Helix*). Besides providing various synthetic chemical macromo-

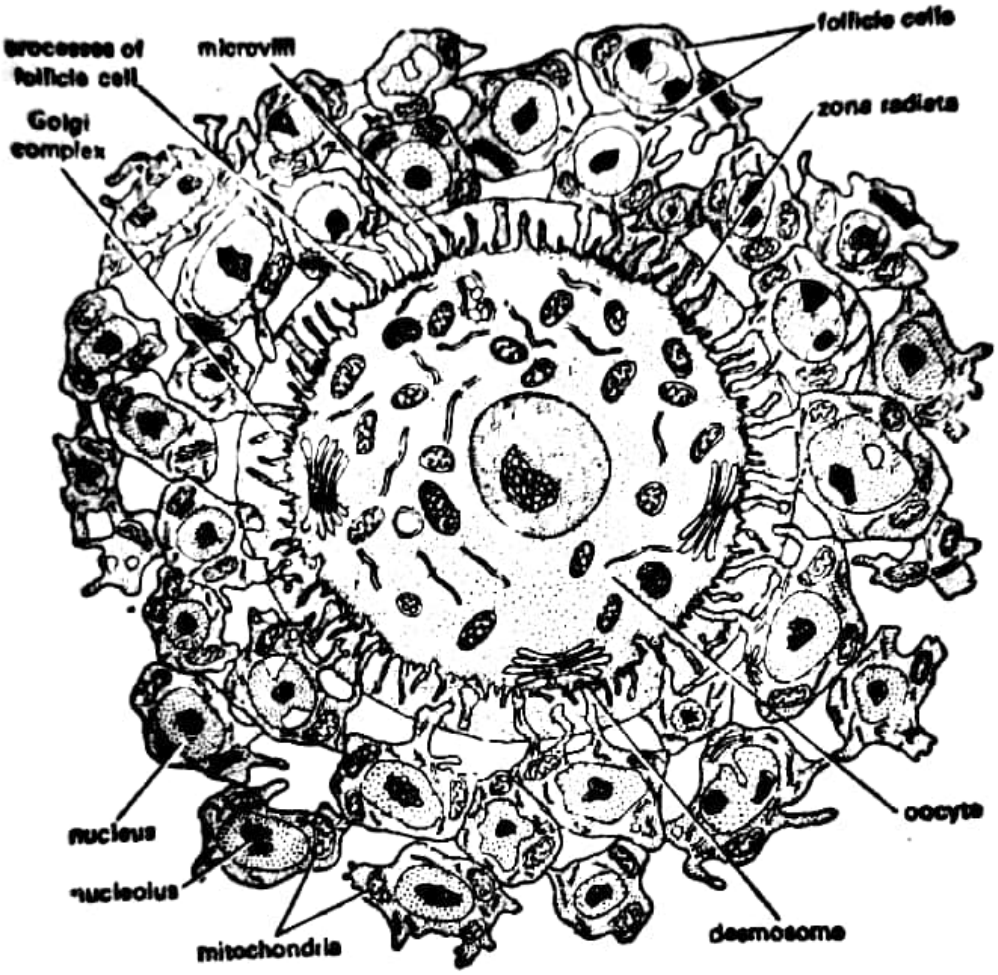

Fig. 3·11. Young oocyte of a mammal surrounded by follicle cells. (After Balinsky, 1970)

lecules such as proteins, lipids, glycogen and nucleic acids (DNA and RNA), the nurse cells provide to the oocyte certain cellular organelle such as ribosomes and mitochondria (Epel, 1973).

C. DIFFERENTIATION

As the oocyte grows and as various inclusions such as proteins, lipids, glycogen, nucleic acids, etc., are synthesized or conserved (after receiving from ovarian follicle cells) in its cytoplasm, a pattern of organization gradually emerges in the cytoplasm of oocyte Each species of egg has its distinctive architecture or organization, which is established by following steps :

(I) BIPOLAR DIFFERENTIATION OR ESTABLISHMENT OF POLARITY OF EGG

The arrangement of various substances (*e.g.*, yolk, RNA molecules, pigment granules) and cellular components in the fully grown

primary oocyte and later, in the egg shows a bipolar differentiation or polarity, *i.e.*, they show an unequal distribution in respect to what may be called the two opposite poles of the egg and in respect to the main axis of the egg—the line connecting the poles. The nucleus of the egg is approximated to one pole of the egg, which has large amount of yolk free cytoplasm and called **animal pole**. The opposite pole is termed as the **vegetal pole**, because the accumulation of yolk at that pole serves for the nutrition of the developing embryo. During maturation phase, the nucleus of oocyte always discharges three polar bodies or polocytes at the animal pole. The main axis of the egg is called **animal vegetal axis**.

The polarity of egg is, thus, apparent in the arrangement of its cytoplasmic inclusions (yolk, etc.). In many eggs the yolk is not evenly distributed, often its density increases from the animal pole towards the vegetal pole, with corresponding structural changes. Besides yolk, certain pigments contained in cytoplasm of egg, also have unequal distribution on both poles. For example, in many amphibians, the animal pole of the egg surface is heavily pigmented, whereas the vegetal region is essentially unpigmented.

Joseph Spek (1934) has provided a very interesting example of bipolar differentiation in the eggs of polychaete *Nereis dumerilii*. In *N. dumerilii* it has been found that during bipolar differentiation of the eggs, the cellular or ooplasmic substances become distributed in such a way that substances of high pH migrated towards the animal pole, while the substances of low pH concentrated towards the vegetal pole. In the egg cells of *N. dumerilii* there is a homogeneously dissolved yellow pigment which is a natural indicator changing colour at about pH 5·4 from yellow to violet from the alkaline towards the acid side. During the bipolar differentiation the pigment is accumulated in the vegetative half of the egg cells, while the animal half becomes colourless (see **Berrill, 1971**).

ORIGIN OF POLARITY OF EGGS

There exists a unresolved controversy regarding the origin of bipolar differentiation or polarity of eggs. Some embryologists claimed that the polarity of the egg may be imposed on it by the direction of flow of nutrient substances during the growth of primary oocyte. It has been mentioned that in molluscs and echinoderms, the vegetal pole of the egg develops from that end of the oocyte by which it remains attached to the wall of the ovary. The nutrient substances, at the expense of which the oocyte grows, presumably enter the ovary from outside, from the body cavity. It would stand to reason, then that greater amounts of yolk might be deposited in the part of the cell nearest to the proximal surface of the ovary, thus

causing this part to become the vegetal pole. This explanation would not hold, however, for oocytes of amphibians or mammals in which the growing primary oocytes are surrounded by follicle cells from all sides. It has been suggested that the course of the nearest blood vessel supplying parts of the ovary with nourishment might cause the parts of the oocyte nearest to the vessel to develop into the vegetal pole. But according to **Child** (1941), the animal pole, as the more active one, should develop from that part of the oocyte which has a better oxygen supply and on this principle the part of the oocyte nearest to a blood vessel should become the animal pole.

According to **Balinsky** (1970), the position of animal and vegetal poles seems to have no connection with the course of the blood vessels, because, polarity is a phenomenon which is found not only in egg cells, but in other cells as well. For example, in epithelial cells their is a distinct difference between their proximal ends (the ends resting on the underlying basement membrane) and their distal ends (which form the free surface of the epithelium). Likewise, in nerve cell the polarity of the cell takes the form of the opposite differentiations of axons and dendrites. Further, in the interior of any cell, the position of the centrosome in respect to the nucleus establishes a general form of polarity, the main axis of which is the line drawn through the centrosome and the nucleus. This polarity has not only affects the distribution of cytoplasmic inclusions (Golgi complex is often found grouped around the centrosome), but, may influence the nuclear structures. For example, in oocyte meiotic prophase, the bivalent chromosomes show a well marked orientation in respect to main axis of cell.

Most embryologists have not reached to definite conclusions concerning the origin of polarity, however, recent investigations with the egg of brown alga, *Fucus* and of *Nereis*, have shown that the polarity of egg is determined by direction of incident light, CO_2 differential factor, or group effect.

(II) ESTABLISHMENT OF SYMMETRY

In addition to an obvious polarity, in many animal eggs, a **bilateral symmetry** is evolved either in a primary oocyte or unfertilized egg (*e.g.*, tunicates and cephalochordates, amphibians, etc.). In an early mammalian oocyte, there exists a polar organization : the nucleus of the oocyte lies nearer to one pole of the egg, the animal pole. A basophilic area called "**yolk nucleus**" containing RNA and mitochondria, lies next to the nucleus, nearer to the vegetal pole, but somewhat to one side of the main axis. In middle-sized oocytes the basophilic area next to the nucleus becomes less conspicuous and the mitochondria are found distributed next to the cortex of the egg.

They become especially numerous on one side of the egg—opposite from where the yolk nucleus was to be seen. The mitochondria are accompanied by large amounts of basophilic granules containing RNA and the ground substance of the cytoplasm is very dense here and rich in proteins. This zone covers about half the surface of the egg, one side of the animal-vegetal axis. The opposite side of the egg cytoplasm is richer in water and contains many vacuoles. The RNA rich side of egg is found to form the dorsal side of the embryo while the side with vacuolated cytoplasm develops into the ventral side of the embryo (**Jonses Seation,** 1950, and **Dalcq**, 1954).

(III) POLARPLASM AND OOPLASMIC SEGREGATION

In the eggs of molluscs, annelids and ascidians, the ooplasm displays some definite organization. For example, in *Dentalium*, the egg is released from the ovary with the germinal vesicle still intact and it proceeds to the meiotic divisions only afterwards. After release from the ovary, the egg partially rounds up but remains slightly flattened from animal to vegetal pole. In a vertical section through the egg, the light area at the vegetal pole (the pole that was attached to the wall of ovary) is seen to be made up of clear yolk-free cytoplasm. This cytoplasm reaches inward to the nucleus and surrounds it, at the outer edges it is continuous with a very thin cortical layer covering the whole of the egg surface. There is also a small patch of clear cytoplasm at the opposite pole; this is the place where the polar bodies will be given off. The rest of the cytoplasm is filled by rather densely packed yolk granules. The yolk-free, unpigmented cytoplasm of vegetal pole is called **vegetal polarplasm.** While, the pigment free **animal polarplasm** is only partly free from yolk. Both types of polarplasm have great significance in development and will be described elsewhere.

In certain eggs (*e.g.*, gastropods), in a primary oocyte or egg, various components of cytoplasm move in such a fashion that there establishes a condition in which different parts of the egg cytoplasm (ooplasm) differ more or less in their chemical composition. This is called **ooplasmic segregation.** Thus, glycogen tends to shift and concentrate towards the animal pole of the egg; RNA concentrates in both polarplasms ; and vitamin C, presumably bound to the Golgi-complex, tends to concentrate into an annular band near the equator of the egg. The displacement and selective accumulation of cytoplasmic components during ooplasmic segregation have been observed among chordates, in ascidians and mammals.

(IV) OOPLASMIC PIGMENTS AND THEIR SIGNIFICANCE IN DEVELOPMENT

During differentiation phase of oogenesis of certain animals

(*e.g.*, sea urchins, ascidians, frog, etc.), there appears some kind of pigment granules in the cytoplasm of primary oocyte. These ooplasmic pigment granules occur in ectoplasm of egg and are found to have no developmental significance, because many animals (*e g.*, *Amphioxus*, *Triturus cristatus*, etc.) lack the ooplasmic pigment granules but have normal development. However, in the eggs in which these pigment granules (yellow in ascidian *Cynthia partia;* red in sea urchin *Paracentrotus lividus* and dark brown in amphibians) occur, there arises a distinct pattern and organization in the ooplasm in respect to them. For example, in frog, the ooplasmic pigments make bipolar differentiation of oocyte well distinct and after fertilization they help in the establishment of bilateral symmetry of fertilized egg.

(V) CORTICAL DIFFERENTIATION

The cytoplasm of growing oocytes and unfertilized eggs of some animals such as echinoderms, molluscs, annelids and amphibians, becomes differentiated into following two regions :

(a) Cortical layer or egg cortex—The egg cortex is composed of three cellular components, namely **ectoplasm, plasma membrane,** and **surface coat**. The ectoplasm is a hyaline layer (a layer of mucopolysaccharides) which is present just beneath the plasma membrane. The formation and the nature of the ectoplasm have been a riddle until it was shown recently to be massed with **microfilaments (Wohlfarth Bottermann,** 1964) which are the linear polymers of **"tensible proteins** (TP)". TP polymers resemble the actin proteins and consequently have been named as 'tectins' by **Mazia** and **Ruby** (1958). However, **Lovtrup** (1974) has coined the term **"tensilins"** for them. Besides microfilaments TP polymers may exist in the cell either as **microtubules** which are the 'one-dimensional' or linear structures or as membranes, which are the 'two-dimensional' structures (see **Lovtrup,** 1974).

Thus, the attachment of microfilaments to the plasma membrane remains instrumental in the formation of the ectoplasm. The presence of microfilaments allows for the deployment of tensile forces by the ectoplasm.

Further, even the egg plasma membrane being a two-dimensional TP polymer contains the tension. Lastly, a thin layear of mucopolysaccharides occurs just outside the egg plasma membrane as most exterior component of egg cortex. It sometimes also contains fibrous proteins and provide protection to the egg. The gelatinous cortex remains in viscous state and its components are not displaceable by cyclosis (cytoplasmic streaming) or centrifugation. The larger cytoplasmic components are either embedded in or attached to the cortex and so remain in fixed position. The egg cortex has a very significant role in the embyrogenesis.

(b) Endoplasm—The interior of egg cytoplasm is occupied by yolk, other inclusions and cellular organalles such as nucleus, mitochondria, Golgi complex, endoplasmic reticulum, etc., and is called **endoplasm**. All the endoplasmic components display a graded distribution along an axis (*i.e.*, animal-vegetal axis) and the nucleus. Further, they are freely movable in natural conditions owing to cytoplasmic streaming or cyclosis.

D. MATURATION OF THE EGG

The phenomenon of maturation begins when the oocyte receives the stimulus which causes rupture of the germinal vesicle and ends with the formation of the second polar body ; it comprises nuclear, cytoplasmic and cortical events, (**Lovtrup, 1974**). As stated in this definition some kind of stimulation is required to initiate the process of maturation. In Vertebrata this is accomplished by pituitary hormones, in some eggs the fertilizatton affords the releasing stimulus and in many other cases contact with the external medium upon shedding suffices to set off the process.

Completion of cell division—It has been mentioned earlier, that during the growth and differentiation periods of oogenesis, the nucleus of primary oocytes remains in a prolonged meiotic prophase during which stage various genetical events such as synapsis, duplication, chiasma formation, and crossing over take place between the homologous chromosomes. After differentiation phase of oogenesis the oocyte nucleus or germinal vesicle resumes meiosis and it reaches the diakinesis stage. Due to some type of stimulation the nuclear membranes of oocyte nucleus break up and the contents of the nucleus become intermingled with the surrounding cytoplasm. The rupture of the nuclear membrane, apart from liberating the chromosomes for the subsequent division, is of great importance because substances such as rRNA, tRNA, mRNA, riboproteins and also certain stimulatory chemical molecules which initiate the formation of microtubules, as mature oocytes normally contain no microtubules but only their monomers or building blocks (**Lovtrup**, 1974), all of which are accumulated in the nucleoplasm during growth phase of oogenesis, are released and become mixed with the rest of the cytoplasm. After the breakdown of the nuclear membrane, the chromosomes which have become greatly contracted and concentrated towards the centre of unbroken germinal vesicle, are carried to the periphery of animal pole of the oocyte. Here, centrioles form a spindle of microtubules which takes up a position perpendicular to the surface of the primary oocyte. Typically, the microtubules of the meiotic apparatus become attached to a narrow region around the animal pole which remains predetermined for the expulsion of polar bodies. Each synapsed homologous

chromosome or tetrad is ultimately separated into two component chromosomes. A bulge now appears at the surface of the animal pole of the oocyte and the outer centriole of spindle with half of the

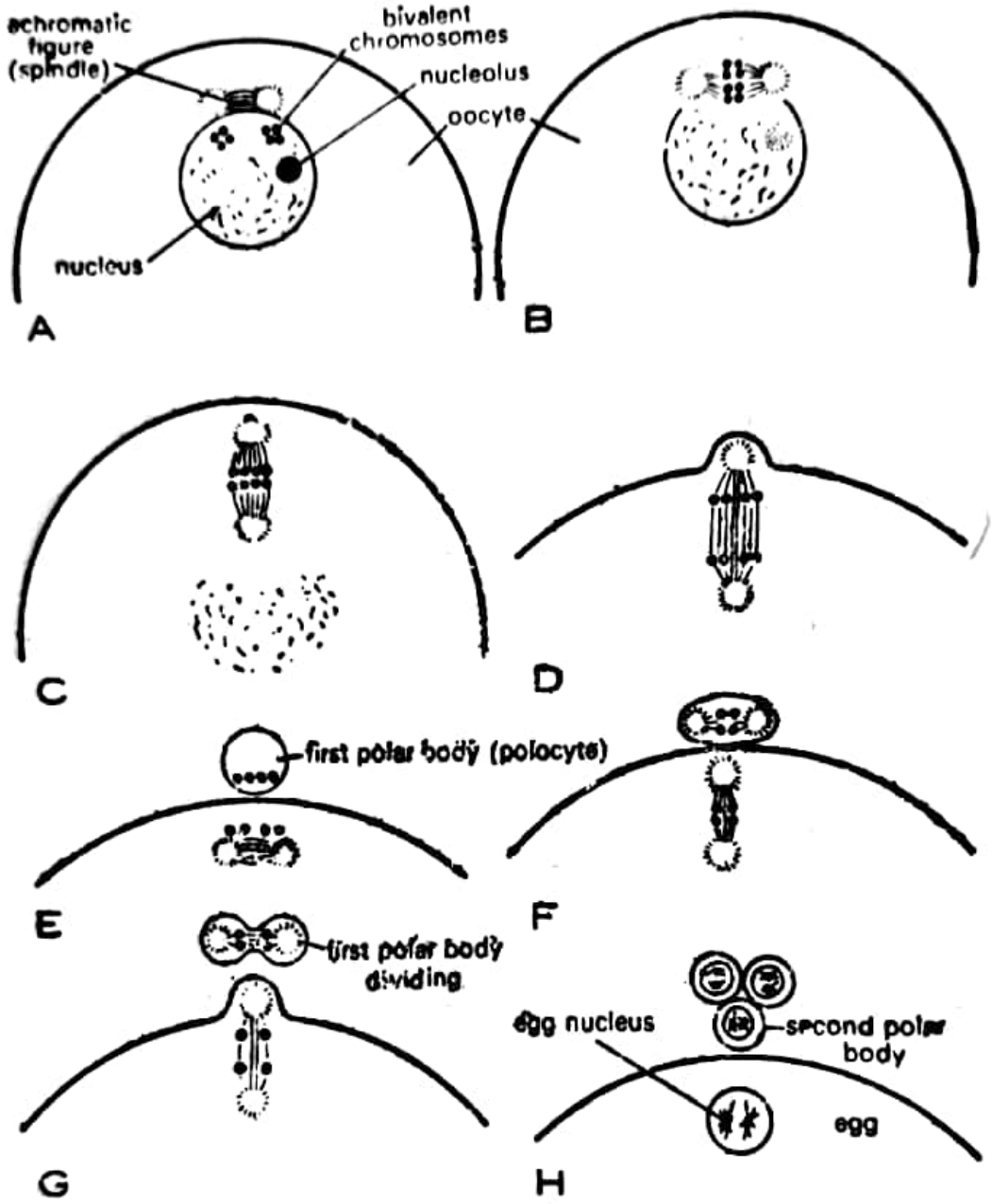

Fig. 3·12. Reduction divisions in an oocyte. A—Oocyte immediately before the meiotic divisions ; B, C, D—First meiotic division ; E—First polar body separated, preparation for second meiotic division ; F, G—Second meiotic division and, simultaneously, division of first polar body ; H—Meiosis completed. (After Balinsky, 1970).

chromosomes (haploid set of chromosomes) enters into this cytoplasmic bulge during the anaphase of first meiosis. The part of the cytoplasm which is bulged out and having a centriole and haploid set of chromosome is pinched off from the rest of the oocyte. Due to this equal nuclear division but unequal cytoplasmic division (cytokinesis) there forms a small-sized, haploid polar body or **polocyte** (the name

polocyte has been given to it, because, it pinches off at the "animal pole" of the egg), and a large-sized cell, called **secondary oocyte** or **ootid**, of the same size as the original cell, the primary oocyte.

The ootid undergoes an equal nuclear division by meiosis II, which is followed by an unequal cytoplasmic division and thus, a haploid **secondary polocyte** and a haploid large-sized **ovum** are formed. The first formed polar body (**primary polocyte**) also divides by meiosis second and two secondary polocytes are formed. Out of these four meiotic products, only the ovum or mature egg remains functional as female gamete and has the capacity to develop into an adult animal, while, three polocytes because have very little cytoplasm, with no food reserve (yolk), lack the capacity to develop further, so later die off.

Significance of unequal cytokinesis during oogenesis—The asymmetrical fractioning of the primary oocyte into three small-sized, haploid polocytes and one large-sized, yolky haploid egg, has great embryological significance. If the equal cytoplasmic divisions of primary oocyte might have been occurred, the reserved food amount (yolk, etc.) would have been distributed equally to the four daughter cells which might proved quite insufficient for the developing embryos. Therefore, unequal cytokinesis allows one cell out of four oogenetic meiotic products to contain most of the cytoplasm and reserve food material which may be quite sufficient for the developing embryo.

Cortical events—During maturation phase of oogenesis, following cortical events occur in the oocyte—1. In many oocytes the surface is rather irregular, but with the onset of maturation it becomes smooth. This change seems to be a prerequisite for fertilization, as **Just** (1939) has generalised that fertilizability is associated with surface smoothness. This phenomenon indicated an increase in **tension**, which may reside in egg membrane or plasma membrane or both. External environment is held responsible to trigger these changes. 2. The cortical granules accumulated in egg cortex (*e.g.*, sea urchin). 3. Microfilamentous activity started. 4. Certain irreversible cortical movements which include translocation of ectoplasm in amphibians occur. 5. Ectoplasm accumulates at animal and vegetal poles in the form of **polar plasms** (see **Lovtrup**, 1974).

FORMATION OF EGG MEMBRANES

The oogenesis is followed by the formation of some protective membranes around the plasma membrane of the egg cell or ovum. In most animals except sponges and coelenterates, the plasma membrane of egg or ovum is surrounded by special protective **accessory envelopes** or **membranes** (Fig. 3.10) which are of following kinds :

1. Primary egg membranes—The primary egg membranes or envelopes are those membranes which are formed around the plasma membrane of egg by the ovum itself. In different animals eggs, the primary egg membranes have different structures and are of following types :

(a) Vitelline membrane—The primary envelope of insects, molluscs, amphibians and birds is called **vitelline membrane**. It contains mucopolysaccharides and certain fibrous proteins. It is usually thin and transparent and is closely applied to the surface of the underlying plasma membrane.

(b) Zona radiata—The primary envelope of shark, some bony fishes, some amphibians and some reptiles have striated appearance and is called **zona radiata**. It represents the degraded microvilli of the growing oocyte. In fishes, perforations in the egg membrane left after the microvilli are withdrawn. These perforations in zona radiata become canals, called **micropiles**, through which the spermatozoa can reach the egg. Bird's eggs lack zona radiata.

(c) Zona pellucida—Zona pellucida is a modified type of zona radiata which is unstriated and is formed by joint efforts of ova and follicle cells. It occurs in mammalian eggs.

(d) Jelly envelope—In echinoderms and many other eggs of marine invertebrates, the primary egg envelop is much thicker structure of jelly coat.

All these primary egg membranes, which have been included under single term **egg membrane** by **Lovtrup** (1974) to avoid confusion, usually adhere closely to the surface of the oocyte, but at a later stage a space filled with fluid may appear between the egg plasma membrane and primary egg membrane. This space is called **perivitelline space.**

Micropyle—In some eggs a small opening, a **micropyle**, is found in the primary egg membrane. The location of the micropyle always bears a definite relation to the animal-vegetal polarity of the egg, being situated either at the presumptive animal, (*e.g.*, Echinoidea, Pisces) or at the presumptive vegetal pole (*e g.*, certain Mollusca). Sperm usually makes its entry inside egg during fertilization through the micropyle. Micropyles arise through inhibition of the growth of egg membrane either because of the attachment to the ovarian wall (vegetal pole) or because a follicle cell establishes contact with the egg surface (animal pole).

2. Secondary egg membranes—The secondary egg membrane or envelope is formed as a basement membrane by a layer of follicle cells surrounding the ovum The secondary envelopes are the chitinous shells surrounding the eggs of insects, ascidians and cyclostomes

and the thin membranes immediately outside the vitelline membrane of the frog's egg. The secondary envelope of eggs of insects, acidians and cyclostomes is called **chorion.** However, the chorion of teleost fishes contains proteins and polysaccharides and it is formed by oocyte, instead of some ovarian tissue.

There are no secondary membranes around the eggs of urodeles, reptiles, and birds. Nor there is a true membranous envelope around the mammalian eggs, but the cells comprising the **ovarian follicle,** within which the mammalian egg lies, are ovarian in origin.

3. **Tertiary egg membranes**—The tertiary egg membranes are formed by the oviduct or other accessory parts of the maternal genital organs while the egg is passing from the ovary to the exterior. Different vertebrates contain different types of tertiary envelopes or membranes.

(a) In oviparous sharks and rays (elasmobranchs), the egg is surrounded by albumen and hard horny capsule of a complicated shape. The shell of capsule is drawn out into long twisted horns which serve to entangle the eggs among sea weeds. The horny egg capsule is secreted by **shell glands** of the oviduct.

(b) The eggs of amphibians are surrounded by a layer of jelly which protects the egg and sometimes serves to make the eggs adhere to one another and to submerged objects such as water plants. The jelly or gelationous covering is secreted as the eggs pass through the oviduct. When the amphibian egg is deposited in water the jelly absorbs water and swells.

(c) The albumen (egg white), shell membranes and outermost calcareous porous shell of the eggs of reptiles and birds, are the best examples of tertiary envelopes. The envelopes are formed by oviducal tissues.

SIGNIFICANCE OF EXTRANEOUS ENVELOPES OF EGG

The extraneous envelopes of eggs of different oviparous vertebrates basically provide the protection to the contents of eggs or developing embryos from different ecological hazards (variable pH, temperature variations, radiations, pollutions, danger of desiccation etc.) and also from mechanical injuries; secondarily, they may prevent self-fertilization as in ascidians, or may provide buoyancy to the eggs as in amphibians and other chordates. Even in viviparous mammals, the extraneous coat such as zona pellucida is found (1) to check polyspermy, *i.e.*, fertilization by more than one spermatozoon ; (2) as a means of preventing egg fusion and (3) as a necessary mean of maintaining normal cleavage of the egg following fertilization. Likewise, the primary egg membranes may be protective, osmoregulative and may even be of great morphogenetic significance.