Module 4

Fertilization

This module will discuss the union of the animal gametes in both male and female parents during fertilization. This will focus also on the events taking place during fertilization in vertebrates and include the fertilization in echinoderms, which is considered a model for studying fertilization.

Learning Objectives: At the end of this lesson, the student should be able to:

1. Discuss the events that happen during the fertilization of the egg cell, including the acrosomal reaction.
2. Explain how polyspermy is prevented.
3. Explain the role of calcium ions during the cortical reaction and how the egg cell is activated to develop.
4. Define capacitation.

Introduction:

The development of an animal begins with the fertilization of gametes from both male and female parents. These gametes as mentioned in the module of gametogenesis are produced in the male and female gonads, the testes and the ovaries, respectively.

Depending on the group of vertebrates (which is the focus of this course), the release of gametes and their transport will vary depending on how the sperm and egg will unite during fertilization.

For most aquatic animals and the anamniotes (vertebrates that do not develop within the amnion), fertilization is **external**. The union of the sperm and the egg takes place outside the females' body. So, the sperms and eggs are released simultaneously in an aqueous environment. A common example of external fertilization is seen in frogs. During this time, the male embraces the female in a position called **amplexus**. You may want watch the video in this link https://youtu.be/JhAlQj_fFuo and see how the eggs are released by the female and just imagine that the sperms are also released at the same time (impossible to see them because they are not seen by the unaided eye 😊). And at that instance of release, external fertilization is happening. There are some fishes wherein after the female have shed the eggs (**spawn/spawning/spawned),** they leave the eggs and allow the "daddy" to fertilize the eggs and care for them (Wow!).

https://phys.org/news/2016-08-female-fish-favor-sperm-males.html

Biologists studying a small, colorful fish in the Mediterranean Sea have discovered a new way in which a female can choose the best father for her offspring.

Biologists have found evidence of "cryptic female choice" involving mechanisms in the reproductive tract that influence which male's sperm fertilize the eggs.

This is what we call now "a choosy lady" 😊

Question 4.1: What could be some drawbacks or disadvantages of external fertilization? List down (you may use bullet points) these disadvantages.

The other kind of fertilization is **internal fertilization.** In this mode, the males generally deliver the sperm cells directly into the female reproductive tract. The moist condition required for sperm motility is provided by the seminal fluid produced by the glands in the male reproductive tract and by the moist tissues in the female tract. This kind of fertilization is practiced by most terrestrial animals like bird, reptiles, and mammals. Sharks and some bony fishes, although are aquatic animals' practice or uses internal fertilization. Internal fertilization is possible by the **intromittent organs** males have. Examples of these organs to deliver the sperms inside the female body are penis (mammals), hemipenis (reptiles), claspers (sharks), gonopodium (bony fishes).

Mammalian penis as seen from a bull. When the penis is not "exposed", it is covered or "hidden" by the foreskin or the prepuce.

https://www.dreamstime.com/photos-images/mammal-penis.html

Hemipenis in a snake. These are evaginations of its cloaca and is used to deliver sperms inside the female's body. These evaginations are also found in other male reptiles.

https://www.reddit.com/r/natureismetal/comments/ctelup/the_hemipenis_of_a_snake/

Claspers are modified pelvic fin skeletons in male cartilaginous fishes including the skates and rays.
https://seaworld.org/animals/all-about/sharks-and-rays/reproduction/

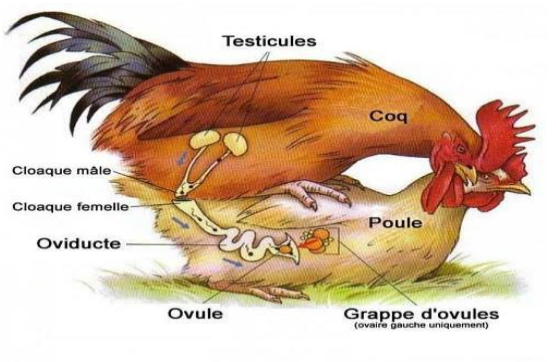
You may want to watch the video taken underwater of a wild encounter of a male and female shark, for possible internal fertilization of the female eggs.
https://www.earthtouchnews.com/natural-world/reproduction/shark-sex-is-fifty-shades-of-rough-video/

Well Endowed Fish Get the Girls…
https://www.livescience.com/249-endowed-fish-girls.html

Male bony fishes have **gonopodium** (white arrows) which are modified anal fins that will be used to deliver the sperm cells inside the female's body for internal fertilization.

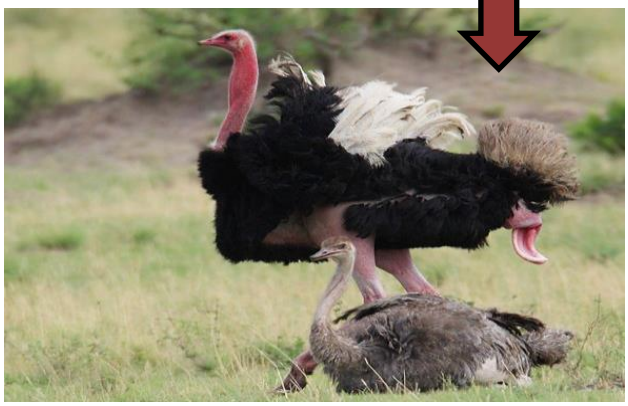
Majority of male birds do not have intromittent organs, but they still practice internal fertilization. But how? They perform the **cloacal kiss.** From this website https://www.quora.com/How-do-birds-have-sex "The cloaca is an internal chamber that ends in an opening, and through this opening, a bird's sex organs — testes or ovaries — discharge sperm or eggs. This same opening also serves a less-sexy purpose: the expulsion of urinary and digestive waste.

During mating seasons, the cloacal openings of both male and female birds swell, protruding slightly outside their bodies. When birds are feeling frisky, they rub their swollen cloacas together. The male's sperm, which has been stored in his cloaca, is deposited into the female's cloaca, where it travels up the chamber and eventually fertilizes an egg".



But a "cloacal kiss" or a series of it do not guarantee that the male who contributes the sperm will be the father of the chicks. Sperm takes time to travel up the oviduct before fertilising the egg. Depending on the species, it may take 30 minutes to days or even weeks before fertilisation actually occurs. Thus many males guard their females after copulation to ensure paternity. But there are ways and means to bypass the males' vigilance if the females so wish...https://besgroup.org/2013/10/21/sex-and-the-birds-1-copulation/

Except



Copulation involves the male balancing on the female's back to pass on his sperms to the female. For land birds like ostriches, emus and curassows, this is not a problem (above). The female can crouch on the ground and remain steady while the male is on her back during copulation. Thus, these land birds, including storks and a few others have a **corkscrew penis that is an erectile expansion of the cloacal wall.** **https://besgroup.org/2013/10/21/sex-and-the-birds-1-copulation/**

Prepared by Margaret L.C. de Guzman. 2020. Department of Biology. College of Arts and Sciences. University of the Philippines Manila.

Question 4.2: What is hermaphroditism? Is it a form of sexual reproduction? Why or why not? (Answer in not more than two sentences).

Question 4.3: Does it entail internal reproduction or external reproduction? (Explain your answer in not more than two sentences)

Once gametes are released from the male and female gonads, their union is the most awaited moment. But of course, this does not happen all the time. Depending on the species, viability for the fertilization of the eggs by the sperms varies. Factors such as time or season, period of ovulation or estrus in non-primate mammals, and of course, proximity of male and female parents, are some of the major factors to be considered for fertilization to occur.

Fertilization is not a single event, instead several steps or processes must occur before the actual union of the male and female nuclei **(pronuclei).** The actual fusion of the nucleus of the sperm cell and the nucleus of the egg cell is the final event of fertilization, commencing the development of a new being. Fusion of the pronuclei restores the diploid chromosome number of the new being of that species as well as allowing the intermingling of the maternal and paternal traits via the chromosomes from both parents.

Generally, in most animals, the steps/components of fertilization would include the following:

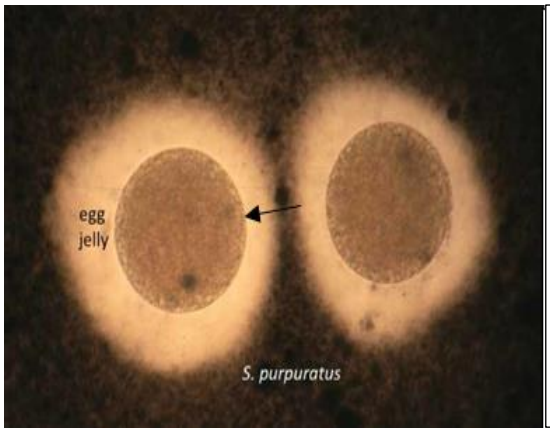
A. Initial membrane contact between egg and sperm
B. Entry of the sperm cell into the egg
C. Prevention of polyspermy
D. Metabolic activation of the egg
E. Completion of meiosis by the egg
F. Formation of the male and female pronuclei
G. Fusion of the pronuclei

Let us look into some of the major details of each event.

But before we proceed, you must be aware that in mammals, as the spermatozoa are released from the male's body, they are not capable of fertilizing an egg. But once they have reached the female reproductive tract, certain maternal tissues make them better to penetrate the membranes surrounding the egg. This phenomenon is called **capacitation of the spermatozoa.** Studies have shown that if mammalian sperms do not undergo capacitation, fertilization is unsuccessful.

A. Initial membrane contact between egg and sperm.

Before the actual fusion of the male and female pronuclei can occur, there are several hurdles and obstacles the sperm cells must encounter and overcome before one sperm cell can penetrate the egg's interior/cytoplasm. In all animals, the egg coverings would be one of the hurdles sperm cells encounter. Aside from being obstacles, the covering adjacent to the egg cell membrane have receptors specific to the species to which the sperms can bind with. Shown in the next pages are egg coverings of selected animals that will be encountered by the sperm cells.



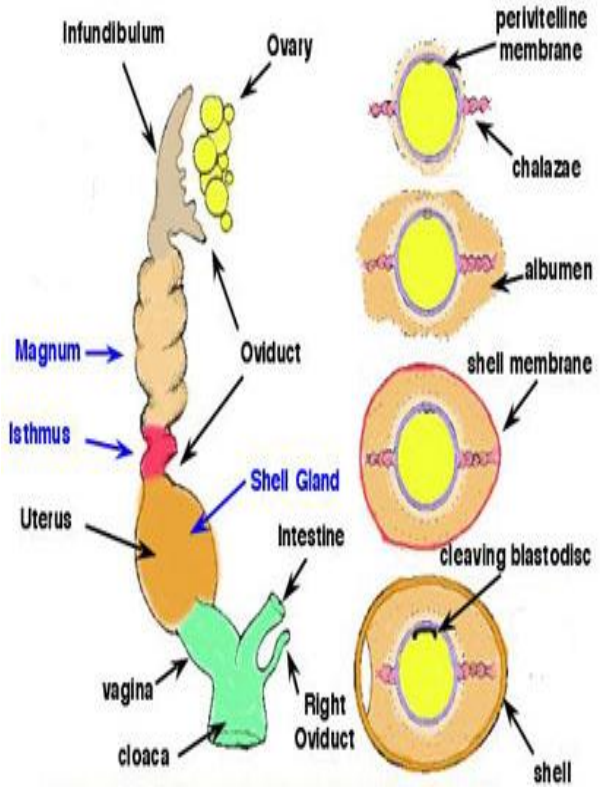
https://onlinelibrary.wiley.com/doi/full/10.1002/mrd.21360

Sea urchins have two non-cellular layers surrounding the egg: just next to the plasma membrane is the **vitelline envelope (black arrow)** provided with species-specific receptors for the spermatozoa; outer to the vitelline envelope is the thick **jelly coat,** which expands when hydrated by water upon release of the eggs to the water medium.

http://www.californiaherps.com/frogs/images/rboyliiamplexsonacr3153.jpg

Like the sea urchin notable in amphibian eggs are the vitelline envelope and the jelly coat which swells upon contact with water.

Later, we will see what happens to the vitelline envelope of both frogs and amphibians when a sperm comes in contact with it.

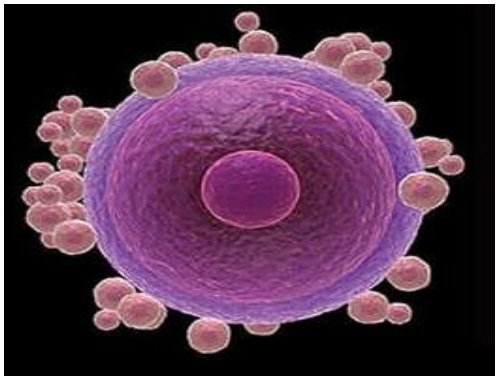


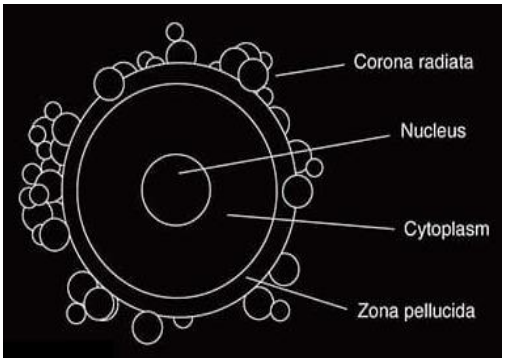
http://people.eku.edu/ritchisong/RITCHISO/avianreproduction.html

"In most birds, only the left ovary and oviduct persist. The ovary enlarges greatly during the breeding season. Active ovaries resemble bunches of tiny grapes -- the developing follicles. The oviduct opens medially to it in a funnel-shaped ostium. Ovulation results in the release of an egg from a mature follicle on the surface of the ovary. The egg, with extensive food reserves in the form of concentric layers of yolk, is picked up by the ostium and ciliary currents carry it into the **magnum** region. Over about three hours the egg receives a coating of albumen.

The egg then passes into the **isthmus**, where the shell membranes are deposited. This takes about one hour. The egg them moves to the **uterus, or shell gland,** where the calcareous shell is added and, in some birds, pigment is added in characteristic patterns. The egg then passes into the vagina and cloaca for laying."

NOTE: Fertilization occurs at the infundibulum where sperm cells are already waiting for the eggs to be ovulated and enter the oviduct.





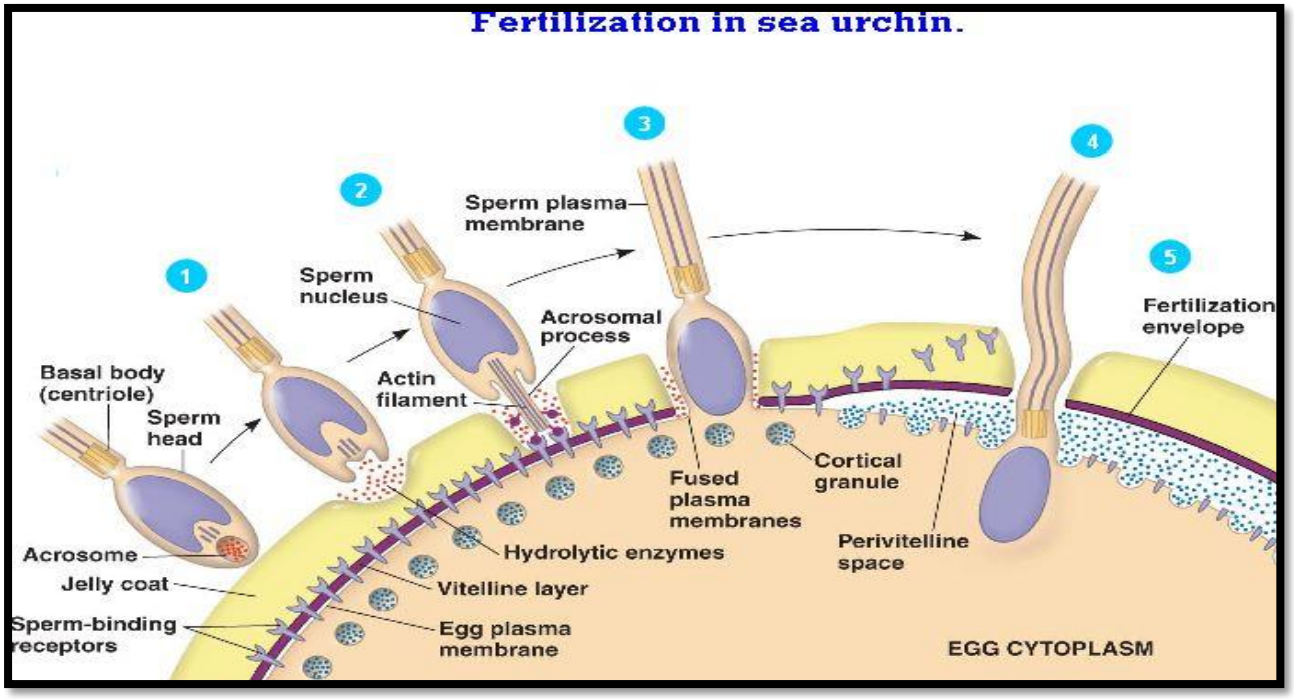
https://www.altrui.co.uk/resources/the-human-egg-cell-explained/

The outermost later of cells surrounding the mammalian egg is the **corona radiata** which is actually two or three layers of follicular cells of the cumulus oophorus. These cells are attached to the zona pellucida. This layer is known to secrete proteins important for the egg cell.

The **zona pellucida** (or egg wall) is made of sulfated glycoproteins with these important roles:

1. Barrier to prevent other species of sperms from gaining access to the egg.
2. Prevents other sperms to enter once a sperm has penetrated the cell membrane
3. Acts as porous substances to allow uterine fluid to enter a zygote in the early cleavage.
4. Prevents immature implantation in the uterine tube

In sea urchins and other invertebrates, when the sperm comes in contact with the jelly coat, the sperm undergoes **acrosome reaction**, wherein the membrane surrounding the acrosome fuse and pores in the membrane enlarge. Shown below is a diagram of the events when the sperm comes in contact with the egg and the evens that follow leading to the entry of the sperm nucleus. We will now look at each step and understand what (generally) happens in each step.



https://bio1152.nicerweb.com/Locked/media/ch47/acrosomal.html

1 **Contact.** The sperm contact with the egg jelly coat, trigger the exocytosis of the sperm's acrosome. The calcium ions from the sea water move into the acrosome, which swells and begin to disorganize the acrosomal membrane and cause the release of hydrolytic enzymes. These enzymes digests or clears the path for the sperm from the jelly coat to the vitelline envelope.

2 **Acrosomal reaction.** Once the jelly coat is digested, actin filaments grow out from the acrosome as **acrosomal process.** This process protrudes out from the sperm head and penetrates the jelly coat. The proteins on the acrosomal process bind to receptors (again as mentioned earlier, are species-specific) in the egg plasma membrane. The protein responsible for the species-specific binding of the acrosomal process to the egg receptors is **bindin**.

3 **Fertilization cone.** The plasma membrane of the egg is covered with microvilli and several of these elongate to surround the head of the sperm. Once the membranes of both sperm and egg fuse, a fertilization cone (FC) is formed that draws in the sperm into the egg. The image on the right was lifted from Solomon, Berg & Martin (2008) where the FC formed by the egg's membrane envelopes surrounds the head of the sperm and by its contraction "swallows" "engulfs" the sperm drawing it inside the egg.

B. Entry of the sperm cell into the egg

4 **Cortical reaction.** Just beneath the egg plasma membrane, in a small region called the **cortex,** are numerous vesicles called **cortical granules.** In a matter of seconds when the sperm binds with the egg membrane, the contents of the cortical granules are released into the space between the plasma membrane and the vitelline layer. The components of the granules (i.e., enzymes and macromolecules) upon their release triggers the **cortical reaction.**

5

Sperm entry. The cortical reaction results to the lifting of the vitelline layer away from the egg and hardens the layer into a protective **fertilization envelope.** The formation of this envelope make certain enzymes cut off the sperm binding receptors thus, discarding the other sperms attached to the eggs surface. The "lucky and strong" sperm gains entry into the egg's interior.

C. Prevention of polyspermy.

More than one sperm fertilizing an egg is a big **NO** in the development of animals designed for sexual reproduction. If there are more than one sperm fertilizes an egg, this is called **polyspermy.** What can happen if there is polyspermy? It can result to offspring with extra sets of chromosomes, most often than not is lethal.

Therefore, nature has two ways of preventing polyspermy in animals. One is called **fast-block** and the second one is **slow-block** to polyspermy.

In the image above of the fertilization of the sea urchin, at step 3, once sperm comes in contact with the egg membrane and fuses to it, the *fast block to polyspermy* is triggered by depolarization of the egg plasma membrane. When an egg is unfertilized, its resting membrane potential is negatively charged relative to the outside (-70mV), but in 2 to 3 seconds after the sperm has fused with it, it becomes electropositive (+10mV). This happens because ion channels in the plasma membrane of the egg opens, allowing positively charged calcium ions (Ca^{2+}) to diffuse cross the membrane and depolarize the egg. This mechanism sorts of "electrocute" the other sperm cells in the vicinity and detaches from the egg 😉. This block is transient or short-lived, a brief moment to allow the egg to prepare for a complete and permanent block, the slow-block to polyspermy. In humans, little is known about the fast-block maybe because, there are fewer sperm cells around the human egg compared to the sea urchins. In fact, no fast block to polyspermy has been identified in mammals (Campbell, et al., 2015).

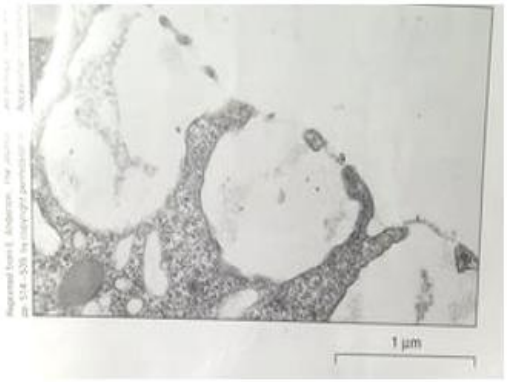
For *slow block to polyspermy*, you can go back to the diagram of the fertilization of the sea urchin and look at step/event #4, the cortical reaction. It is in this step/event that slow block to polyspermy happens. What happens in the cortical reaction that can lead to blocking permanently additional sperms to enter the egg cell once a sperm cell has come in contact with the egg's surface?

a. Once a sperm is bound to the receptors on the vitelline envelope, a number of signal transduction pathways in the egg are activated.
b. These pathways cause the endoplasmic reticulum to release calcium ions into the cytoplasm.
c. This rise in the cytosolic calcium ions causes the cortical granules to release enzymes, various

proteins and other substances by exocytosis into the space between the plasma membrane and the vitelline envelope.

d. The enzymes break the link between the plasma membrane and the vitelline envelope causing them to separate.
e. In addition, the space between the two coverings is further raised or elevated because the other substances from the granules increases the osmotic pressure, thus causing influx of water from the surrounding to the space
f. A fertilization envelope (step #5) is formed, which is a hard covering, preventing entry of additional sperm. In mammals, a fertilization envelope is not formed, instead, the enzymes released during exocytosis of the cortical granules hydrolyze the sperm receptors on the egg's zona pellucida so that no additional sperm bind to them. This is called the **zona reaction** (Carlson, 1994).

Below is an image of the cortical reaction in a sea urchin egg.



The cortical reaction

(Lifted from Solomon, Berg & Martin, 2008). A TEM image of three cortical granules undergoing exocytosis in the plasma membrane of a sea urchin egg shortly after fertilization. The release of the contents of the cortical granules initiates the slow block to polyspermy. (From E. Anderson, Reprinted from *The Journal of Cell Biology*, 1968, Vol. 37, pgs 514-539, by copyright permission of the Rockefeller University Press)

D. Metabolic activation of the egg.

Once a sperm cell has penetrated the egg, it rapidly intensifies the egg's respiration and metabolism. Just so you know, once a human egg is ovulated, it has 24 hours viability. Meaning it can be fertilized within that 24 h period and be activated to proceed to the next stage of development. If that ovulated egg is not fertilized in 24 h, it will "die" that results to no implantation in the uterus of a fertilized egg, leading to the shedding of the endometrium as menses of the menstrual phase of the uterine cycle.

What is the explanation of the activation of the egg once a sperm has entered the egg cell? The calcium ions released from the endoplasmic

reticulum to the cytoplasm does not only initiate the cortical reaction but also **triggers** the **activation program**. This program involves a series of metabolic changes within the egg, that includes:

a. Increase in aerobic respiration
b. Maternal enzymes and proteins become active (which were already present in the egg prior to fertilization)
c. More proteins are synthesized
d. Egg nucleus is stimulated to complete meiosis.

Question 4.4: What is parthenogenesis? What form of reproduction is it? How about the fertilization of the eggs, is it external or internal or none? Can a new being develop from this process, how?

E. Completion of meiosis by the egg

As mentioned previously, the cortical reaction for one stimulates the egg to complete the arrested meiosis II. For sea urchin eggs, they have already completed meiosis II when they are released from the female body. But for other species, the eggs released from the ovary are at varying stages of meiosis II before fertilization. But once the head of the sperm has penetrated the egg coverings and has entered the inside of the egg, the arrested meiotic stage is lifted and proceeds to finish meiosis.

In humans, the ovulated secondary oocyte (1N, 2C) is arrested at metaphase II. But once it is fertilized, the rest of the second meiotic division is completed making the egg cell completely haploid. Note that, as the secondary oocyte completes meiosis II, the second polar body is released to the perivitelline space.

F. Formation of the male and female pronuclei

The chromosomal materials of the egg then become surrounded by a **pronuclear membrane** (possibly derived from the endoplasmic reticulum) forming the **female pronucleus**.

For the sperm nucleus, once its head has entered the egg cytoplasm, the permeability of its nuclear membrane is increased leading to its disintegration. This then makes the sperm's chromatin to spread out in the egg's cytoplasm (**decondensation**). Later on, a new nuclear membrane will form around the chromatin and is now properly called the **male pronucleus.** The flagellum/tail and the mitochondria are either left outside or when they have come inside with the nucleus, they detach in the cytoplasm of the egg cell and degenerate. The centrioles of the sperm on the other hand, persist with the nucleus as this will guide the sperm in moving towards the female pronucleus by forming the **sperm aster.**

G. Fusion of the male and female pronucleus.

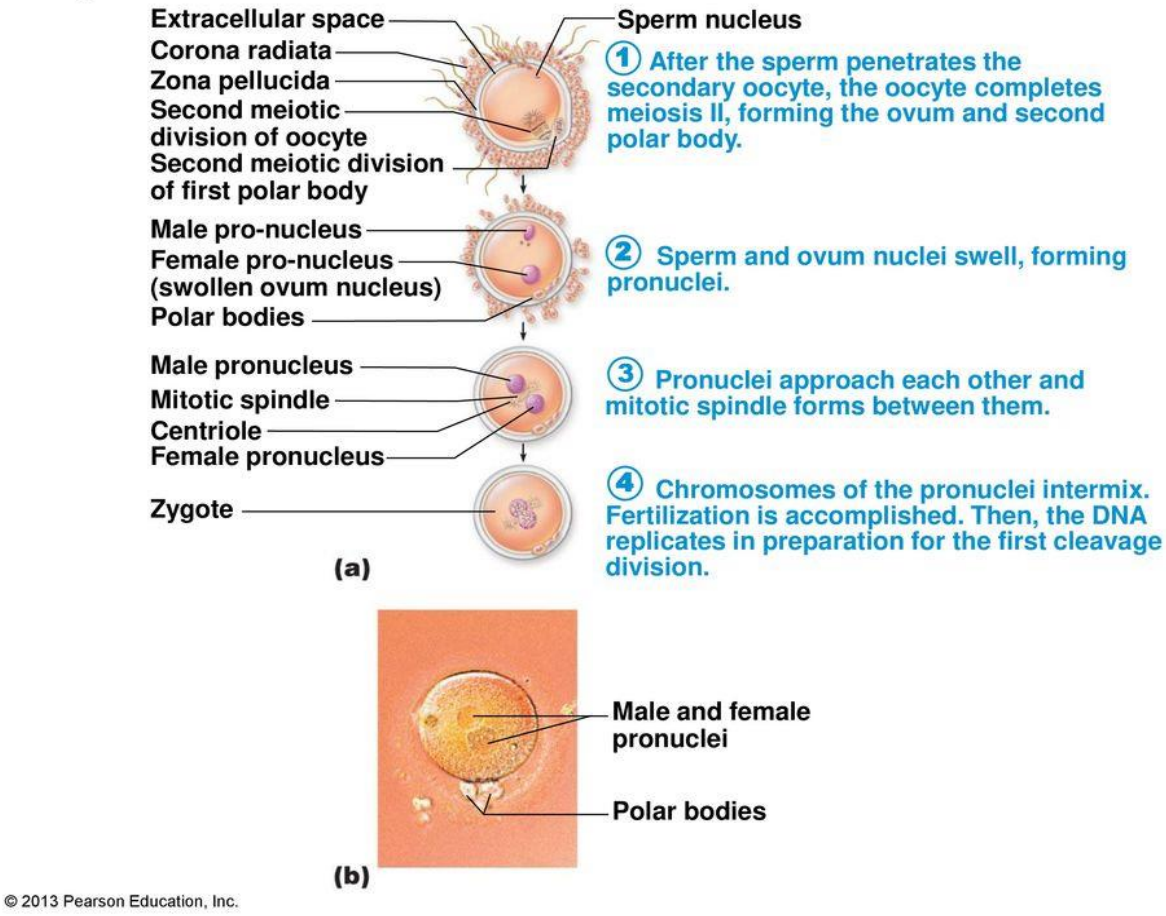
Both pronuclei grow further as they move closer to each other. When both pronuclei come in contact with each other, their membranes fuse, making the maternal and paternal chromosomes enclosed in a single membrane. This event is called **pronuclear fusion.** Right after the fusion, the haploid maternal chromosomes and the haploid paternal chromosomes duplicate/replicate/synthesize their DNAs in preparation for the first cleavage division. It is in the interphase of the cell cycle, when the DNAs are replicated and by the time the chromosomes are aligned in the metaphase plate, **the process of fertilization is completed (yehey!!!!)**, and the fertilized egg is then called a **zygote**.

What is accomplished by fertilization?

1. It stimulates the egg to complete the second meiotic division.
2. It restores the normal diploid number of chromosomes (2n) of the species.
3. The sex of the future embryo is determined by the chromosomal complement of the spermatozoon:
    a. Spermatozoon has 22 autosomes and X chromosome = female (XX)
    b. Spermatozoon has 22 autosomes and Y chromosome = male (XY)
4. The mingling of the maternal and the paternal chromosomes, the zygote will have a mixture of the reassortment of traits of the parents, which is important for the survival of any species.
5. It activates the egg metabolically, thereby causing it to proceed with the next stage of embryonic development, cleavage.

Shown in the next page is a graphical summary of the process of fertilization in mammals.

Figure 28.3 Events of fertilization.



A summary of the events of fertilization in mammals
https://slideplayer.com/slide/12116673/

BOOK REFERENCES:

1. Campbell NA, Reece JB, Urry LA, *et al.* 2015. Biology A Global Approach. Pearson Education, Ltd.
2. Carlson B.M. 1994. Human Embryology and Developmental Biology. Mosby.
3. Carlson, B.M. 1996. Patten's Foundation of Embryology. 6th ed. McGraw Hill Book Company.
4. Solomon EP, Berg LR and Martin DW. 2008. Biology. 8th ed. Thomson Brooks/Cole.
5. Wilt, F & S. Hake. 2004. Principles of Developmental Biology. W.W. Norton & Company